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CHARACTERIZATION OF THE SPONTANEOUSLY DIABETIC BB WISTAR RAT

DISSERTATION

Presented in Partial fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

James Roscoe Wright, Jr., B.S., M.A., M.D.

* * * * *

The Ohio State University
1995

Dissertation Committee: Approved by

C.C. Capen
D.A. Senhauser
H.M. Sharma
R.E. Stephens
A.J. Yates

Advisor
Department of Pathology
To Allan J. Yates, M.D., Ph.D.

He chased me around the world until I agreed to finish this!
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VITA

September 21, 1955 ....... Born - Huntsville, Alabama
1977 ....................... B.S., Ohio State University, Columbus, OH
1977-1979 .................. Graduate Research Associate
                         Depts. of Surgery & Pathology,
                         Ohio State University
1984 ........................ M.A., Ohio State University
1984 ........................ M.D., Ohio State University
1984-1988 ................... Resident, Dept. of Pathology
                         Barnes Hospital, Washington
                         University, St. Louis, MO
1985-87 ..................... NIADDKD Trainee (Diabetes)
                         Washington University
1988-1990 ................... Fellow, Pediatric/Perinatal
                         Pathology, IWK Children's
                         Hospital & Grace Maternity
                         Hospital, Dalhousie
                         University, Halifax, Nova
                         Scotia, Canada
1989-1993 ................... Clinical Assistant,
                         IWK Children's Hospital
1989-1994 ................... Assistant Professor of Pathology
                         Dalhousie University
1990-present ............... Assistant Professor of Surgery
                         Dalhousie University
1993 ........................ Pathologist, IWK Children's
                         Hospital & Grace Maternity
                         Hospital
1994-present ............... Associate Professor of Pathology
                         Dalhousie University
PUBLICATIONS


Wright JR Jr: review of Jonas Rishel's The Indian Physician, containing a new system of practice, founded on medical plants: together with a description of their properties, localities, and method of using and preparing them... (New Berlin, PA: Joseph Miller, 1828; photostatically reprinted by the Ohio State University Libraries Publication Committee, 1980). Ohio State University College of Medicine Journal 31: 17, 1981.


FIELDS OF STUDY

Major Field: Pathology

Studies in Experimental Pathology (Allan J. Yates, M.D., Ph.D.)
Islet Transplantation (Paul E. Lacy, M.D., Ph.D.)
Immunology (Emil R. Unanue, M.D.)
Anatomical Pathology (John Kissane, M.D.)
Pediatric Pathology (Blaise E. Favara, M.D.)
Placental Pathology (Stephen A. Heifetz, M.D.)

Minor Field: History of Medicine (John C. Burnham, Ph.D.)
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xxvi
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CHAPTER I
INTRODUCTION

The validity of studying animal models for human disorders is a longstanding dilemma for the medical scientist. The obstacles for studying factors involved in the pathogenesis of a disorder in a population of human subjects are large. The importance of both genetic predisposition and environmental factors to the manifestation of many disorders is unquestionable. Since it is ethically unacceptable to control the genetic background of human subjects and since it is exceptionally difficult to control chronic environmental conditions, the usefulness of human prospective studies is severely restricted. Furthermore, the cost of prospective studies is immense. On the other hand, retrospective epidemiological studies are relatively inexpensive but, unfortunately, must rely on information from numerous sources of uncontrollable reliability. In addition, selection of a homogenous group of appropriate human subjects is not an easy task.

Upon superficial examination, many of these important variables appear more easily controlled by utilizing animal models. Unfortunately, any animal model introduces a new set of uncontrollable variables. Rarely is any model for a
human disorder a normal animal with a single variable changed. Frequently, the metabolic disorder that precipitates the overt symptoms of the desired disorder may be either the cause or the result of other underlying disorders. Even when the abnormalities in the animal model appear identical to those of the human disease, there is no reason to believe that the underlying defects causing those abnormalities are related. In other words, similar abnormalities do not necessarily imply a similar etiology or pathogenesis.

This makes the choice of an appropriate animal model for an experimental study as important as the overall experimental design. One would like to choose the model that most closely approximates the human disease. Unfortunately, the information necessary to make this decision is not always readily available. The goal of my dissertation is to characterize several aspects of the spontaneously diabetic BB Wistar rat, a model for insulin-dependent (juvenile-onset) diabetes mellitus (IDDM). This will permit future investigators to determine whether this model is appropriate for the objectives of their studies.
BACKGROUND INFORMATION

A. History of the BBW Rat

The spontaneously diabetic Bio Breeding Wistar (BBW) rat was discovered by chance in 1974 at the Bio Breeding Laboratories of Canada. It was noted that the high mortality rate within an outbred colony of Wistar rats at the facility was also associated with persistently wet bedding. After the possibility of a defective watering system was ruled out, other possibilities were explored. The presence of polyuria and the discovery of both glucose and ketones in the urine was definitive for the diagnosis of diabetes mellitus. Because of the obvious importance of maintaining and studying the colony, and because of the expenses associated with that task, funding was requested from the Canadian Medical Research Council (MRC) to support the endeavor. After receiving MRC support, studies on the BBW rat were expanded. The pilot studies were performed through the collaborative efforts of Drs. Nakhooda, Wei, and Marliss at the University of Toronto, Dr. Like at the
University of Massachusetts, and Dr. Chappel at the Bio Breeding Laboratories (Nakhooda et al., 1977; Nakhooda et al., 1978).

These pilot studies resulted in several very important observations that suggested that the BBW rat was like no other model of IDDM yet studied. First of all, the diabetic syndrome in the BB Wistar rat was characterized by variable degrees of hyperglycemia, glycosuria, hypoinsulinemia, hyperglucagonemia, and ketoacidosis. These symptoms could be minimized by proper insulin treatment. Untreated BBW rats could be divided into three groups based on level of ketosis: 1) severely ketotic (total blood ketone body levels between 6-13 mM), 2) moderately ketotic (1-5 mM), and 3) stable (<1 mM). Severely ketotic rats showed rapid weight loss and dehydration in one to six days. Moderately ketotic rats gradually lost weight over 15 days but maintained marked polyuria and glycosuria. Stable rats maintained weight, polyuria, and glycosuria in excess of 40 days.

Second, light microscopic examination of the pancreas from stable and early ketotic rats indicated that islets were small, frequently inflamed, and had reduced numbers of beta cells showing variable degranulation. Islets from severely ketotic rats were exceedingly small and rare; beta cells were seldom present. From these findings Nakhooda et al. (1977) concluded that a widespread insulitis was responsible for active beta-cell destruction. A milder form of this
insulitis was also observed in a small number of BB Wistar litter mate controls. Further study demonstrated that most of these rats were "chemically diabetic", i.e. they had abnormal glucose tolerance tests but no hyperglycemia or glycosuria. It was also determined that abnormal glucose tolerance preceded the onset of overt diabetes in most of the BBW diabetic rats (Nakhooda et al., 1978). Although complete remission of the diabetic syndrome was occasionally seen, the diabetic state was usually severe and permanent (Nakhooda et al., 1978). Like reported that only 6 spontaneous cures were observed in more than 1000 diabetic animals studied (Like et al., 1982).

Continued interest by the Canadian Government, and financial difficulties at the Bio Breeding Laboratories resulted in the transfer of the colony to the Animal Resources Division of Health and Welfare Canada in December of 1977 and January of 1978 (Chappel and Chappel, 1983). New maintenance techniques, such as monogamous sibling mating, implemented following the transfer increased the average incidence of diabetes within the litters of diabetic matings from 20-25% (average onset at 63 days of age) to 70-80% (average onset at 85 days of age). Furthermore, the number of unproductive matings was decreased from 50% to 15%. Unfortunately, sibling mating also resulted in smaller litters and numerous developmental abnormalities and had to be discontinued after about eight generations. Technically
speaking, these BB Wistar rats were still outbred, but skin
graft rejection studies indicated that these animals were
for all practical purposes inbred.

At the time our studies were performed, the overall
incidence of hyperglycemia in these animals was about 60%
with an average postnatal time of onset of 85 days, but this
pattern was quite variable. Furthermore, the number of
pups/litter varied from 6-15 depending on whether one or
both parents were diabetic. When both parents were
diabetic, fecundity was decreased but incidence within the
litter was increased (Personal Communication, P. Thibert).

Following the transfer to Health and Welfare, the
animals became more readily available to the scientific
community. By 1979, spontaneously diabetic BB Wistar rats
were available from:

Pierre Thibert, D.V.M.
Chief, Animal Resources Division
Health Protection Branch
Health and Welfare of Canada
Sir Frederick G. Banting Research Centre
Tunney's Pasture, Ottawa, Ontario
Canada, K1A 0L2
(613) 957-0865

A breeding colony of BB Wistar rats was initiated in
the Pathology Department at the Ohio State University in
April of 1979. Animals were shipped from Ottawa by
commercial airlines to Columbus via Pittsburgh. Many of the
animals in our first few shipments from the parent colony in
Ottawa died of pneumonia acquired in transit or while housed
in a non-barrier facility in Graves Hall. The colony was later moved to a semi-barrier facility in Wiseman Hall in July of 1979 to decrease the incidence of pulmonary infections. A necropsy study was initiated to assure quality control within our colony and was expanded following a series of interesting findings. This study is based in part on those findings.

In the early 1980s, the University of Massachusetts Medical School received a contract from the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) to breed and distribute diabetes-prone and diabetes-resistant BB Wistar rats to eligible investigators. In Worcester, several inbred lines were developed and characterized. BB/Wor rats became available from:

Dennis L. Gubereshki, BB\Wor Project Administrator
Department of Pathology
University of Massachusetts Medical School
55 Lake Avenue North
Worcester, Massachusetts 01605
(508) 856-3366

Since that time, the BB Wistar rat has become one of the most extensively studied animal models for diabetes. We estimate that over a thousand papers have been published using this model since its development. Many of these findings have been described in a series of comprehensive review articles (Like et al., 1982; Marliss et al., 1982; Like and Rossini, 1984; Rossini et al., 1985; Eisenbarth, 1986; Mordes et al., 1987; Kastern et al., 1990; Scot et
B. Other Models of Diabetes

Numerous spontaneous models for diabetes mellitus have been studied in the past 50 years (Hansen and Hansen, 1990). Rather than reviewing each model separately, I have constructed the tables 2.1, 2.2 and 2.3 which permit comparison of salient features. These tables are the result of an extensive literature review but are still not exhaustive. Since many of these models were characterized 25 or more years ago, mechanized information searches are of limited value in accessing some of this information.

Table 2.1 shows 15 selected spontaneous rodent models for diabetes mellitus. Although other models exist (many are hybrids resulting from cross breeding these models), these 15 are the best known and most frequently utilized. It is useful to describe each rodent as a model for either IDDM or non-IDDM (NIDDM). In this present study we are interested in the models that are similar to human IDDM, but we will briefly examine the models for NIDDM. In general, NIDDM models are obese, hyperglycemic, nonketotic, and have elevated serum insulin (Table 2.1). The pancreatic islet morphology of these models is similar to that seen in human
NIDDM. Most of these show beta cell hyperplasia and an absence of definitive beta cell degranulation, necrosis, or insulitis (Table 2.2). Models of NIDDM are reviewed in the National Research Council's *Institute of Laboratory Animal Resources (ILAR) News* in the Summer 1990 issue (volume 32, number 3).

Six models are similar to human IDDM. These are the Chinese hamster, the South African hamster, the New Zealand White Rabbit, the non-obese diabetic (NOD) mouse, the Long-Evans Tokushima Lean (LETL) rat, and the BB Wistar rat. They share certain metabolic features including hyperglycemia, the absence of obesity, and decreased plasma insulin. Ketoacidosis, an important feature of human IDDM, is seen only in the Chinese hamster, the South African hamster, the LETL rat, and the BB Wistar rat. The typical human IDDM pattern of islet morphology is B cell degranulation, glycogen deposition, and necrosis. Only the New Zealand White Rabbit differs from this general pattern (Table 2.2). Insulitis, a transient feature of the human disease, is seen only in the BB Wistar rat, the LETL rat, and the NOD mouse. Beta cell hyperplasia is not seen in either human IDDM or the IDDM models (Table 2.2). Models of IDDM are reviewed in the Winter 1993 issue of *ILAR News* (volume 35, number 1).

There are certain inherent advantages of the BBW rat as a model for IDDM. First, it is one of only three models
that develops an insulitis at the onset of the diabetic state. Second, it is a much larger animal than the NOD mouse, the Chinese hamster, or the South African hamster. Animal size is important for many types of experimentation. Third, it is much more widely available and better characterized than the LETL rat. Finally, the BBW rat has a gentle temperament compared to the Chinese hamster, an animal known for its ferocious nature. For these reasons, the BB Wistar rat is one of the most promising model for IDDM research. Therefore, I have characterized various previously unexamined features of this model.
# TABLE 2.1 COMPARISON OF SELECTED SPONTANEOUS RODENT MODELS FOR DIABETES MELLITUS

## METABOLIC CHARACTERISTICS

<table>
<thead>
<tr>
<th>MODEL</th>
<th>TYPE OF DIABETES</th>
<th>GENETICS</th>
<th>LETHALITY</th>
<th>OBESITY</th>
<th>HYPERGLYCEMIA</th>
<th>KETOACIDOSIS</th>
<th>PLASMA IRI</th>
<th>INSULIN RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese Yellow Mouse</td>
<td>NIDDM</td>
<td>autosomal dominant</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>-</td>
<td>-</td>
<td>△</td>
</tr>
<tr>
<td>Obese Hyperglycemia (ob/ob)</td>
<td>NIDDM</td>
<td>autosomal recessive</td>
<td>-</td>
<td>△</td>
<td>transient</td>
<td>-</td>
<td>1/normal</td>
<td>△</td>
</tr>
<tr>
<td>Diabetic (db/db) Mouse</td>
<td>NIDDM</td>
<td>autosomal recessive</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>New Zealand Obese (NZO) Mouse</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>-</td>
<td>△</td>
<td>△</td>
<td>-</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Japanese KK Mouse</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>-</td>
<td>△</td>
<td>transient</td>
<td>-</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Spiny Mouse (Acomys cahirinus)</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Egyptian Sand Rat (Pseudomys obesus, Desert Rat)</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>transient</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Spontaneously Diabetic Wistar Rats (Japan)</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Zucker &quot;Fatty&quot; rat</td>
<td>NIDDM</td>
<td>autosomal recessive</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Man</td>
<td>NIDDM</td>
<td>polygenic</td>
<td>-</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Chinese Hamster (Cricetulus griseus)</td>
<td>IDDM</td>
<td>polygenic</td>
<td>-</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>1/normal</td>
<td>△</td>
</tr>
<tr>
<td>South African Hamster (Hystromys albicaudatus, white tailed rat)</td>
<td>IDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>New Zealand White Rabbit</td>
<td>IDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>BB Wistar (BBW) Rat</td>
<td>IDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Non-obese diabetic (MOD) mouse</td>
<td>IDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Long-Evans Tokushima Lean (LETL rat)</td>
<td>IDDM</td>
<td>polygenic</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>Man</td>
<td>IDDM</td>
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<td>△</td>
<td>△</td>
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<td>△</td>
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</tr>
</tbody>
</table>
TABLE 2.2 COMPARISON OF SELECTED SPONTANEOUS RODENT MODELS FOR DIABETES MELLITUS

**PANCREATIC BETA CELL MORPHOLOGY**

<table>
<thead>
<tr>
<th>MODEL</th>
<th>B CELL HYPERPLASIA</th>
<th>B CELL DEGRANULATION</th>
<th>B CELL GLYCOGEN</th>
<th>B CELL NECROSIS</th>
<th>INSULIN</th>
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<tbody>
<tr>
<td>Obese Yellow Mouse</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Obese Hypertensive Mouse</td>
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<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diabetic Mouse</td>
<td></td>
<td>transient</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>New Zealand Obese Mouse</td>
<td>+</td>
<td></td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Japanese KK Mouse</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spiny Mouse</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Egyptian Sand Rat</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Spontaneous Diabetic Zucker Rat (Jazan)</td>
<td>?</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zucker &quot;lady&quot; rat</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>Man (NOD)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chinese Hamster</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>South African Hamster</td>
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<tr>
<td>NOD Mouse</td>
<td>+</td>
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<tr>
<td>BB Wistar Rat</td>
<td>+</td>
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<tr>
<td>LETL rat</td>
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<td>+</td>
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<tr>
<td>Man (DDM)</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>
# TABLE 2.3 COMPARISON OF SELECTED SPONTANEOUS RODENT MODELS FOR DIABETES MELLITUS

## MAINTENANCE INFORMATION

<table>
<thead>
<tr>
<th>MODEL</th>
<th>INSULIN REQUIRED FOR MAINTENANCE</th>
<th>IMPAIRED BREEDING</th>
<th>IMPAIRED IN LITTER</th>
<th>AGE OF ONSET</th>
<th>COMPLETE PATHOLOGY PROFILE AVAILABLE</th>
<th>LIFE SPAN</th>
<th>BODY WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese Yellow Mouse</td>
<td>-</td>
<td>+</td>
<td>50%</td>
<td>puberty</td>
<td></td>
<td>max &gt; 18 mo</td>
<td>&lt; 80g</td>
</tr>
<tr>
<td>Obese Hyperglycemic (ob/ob) Mouse</td>
<td>-</td>
<td>+</td>
<td>25%</td>
<td>4-6 wk.</td>
<td></td>
<td>15 mo.</td>
<td>&lt; 120g</td>
</tr>
<tr>
<td>Diabetic (db/db) Mouse</td>
<td>-</td>
<td>+</td>
<td>25%</td>
<td>5-6 wk.</td>
<td>partial</td>
<td>3-6 mo (12 mo. max)</td>
<td>45g</td>
</tr>
<tr>
<td>New Zealand Obese (NZO) Mouse</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>partial</td>
<td>?</td>
<td>&lt; 72g</td>
</tr>
<tr>
<td>Japanese KK Mouse</td>
<td>-</td>
<td>-</td>
<td>50-90%</td>
<td>6-8 wk.</td>
<td></td>
<td>?</td>
<td>&lt; 45g</td>
</tr>
<tr>
<td>Spiny Mouse</td>
<td>-</td>
<td>-</td>
<td>15%</td>
<td>6-17 mo.</td>
<td>?</td>
<td>?</td>
<td>60-85g</td>
</tr>
<tr>
<td>Egyptian Sand Rat</td>
<td>-</td>
<td>?</td>
<td>diet dependent</td>
<td>?</td>
<td></td>
<td>max. &gt; 32 mo</td>
<td>118-225g</td>
</tr>
<tr>
<td>Spontaneously Diabetic Wistar Rats (Japan)</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
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<tr>
<td>Chinese Hamster</td>
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<td>max. &gt; 23 mo</td>
<td>22-35g</td>
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<td>South African Hamster</td>
<td>-</td>
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<td>-4 mo.</td>
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<td>14 mo. max.</td>
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<td>New Zealand White Rabbit</td>
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<td>?</td>
<td>19%</td>
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<td>NOD Mouse</td>
<td>-</td>
<td>?</td>
<td>&gt; 90% female</td>
<td>7 mo.</td>
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<tr>
<td>BB Wistar (BBW) Rat</td>
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<td>+</td>
<td>70%</td>
<td>2-4 mo</td>
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<td>&lt; 400g</td>
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<td>LETL Rat</td>
<td>+</td>
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<td>14-64%</td>
<td>2-5 mo</td>
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CHAPTER III
METHODOLOGY

A. Colony Husbandry

A breeding colony of BB Wistar diabetic (BBWd) rats was established in the Pathology Department of the Ohio State University from the parent colony at the Health Protection Branch of the Canadian Government. Animals were shipped by commercial airlines from Ottawa to Columbus, Ohio where they were originally housed in a standard animal housing facility, and later in a semi-barrier housing facility.

The barrier facility was constructed with glazed tile walls and epoxy-terrazo floors. The room was maintained under slight positive pressure at 22°C and was supplied with fresh air passed through high efficiency particle fitters at a rate of 16 air changes per hour. Humidity varied between 45 and 55% and a constant photoperiod was maintained with a 12 hour light-dark cycle.

Rats were housed as mating pairs in 27.9 x 48.3 x 17.8 cm. polycarbonate cages with sanitized hardwood chip bedding (Sani-Chips, FJ Murphy Co., Moonachie, NJ). Cages were sanitized at 82.2°C and changed twice each week. All rats had free access to pellet rat chow (Purina Laboratory Chow,
Ralston Purina Co., Richland, Indiana) and water. Water bottles were acid cleaned weekly and fresh tap water was supplied daily.

All rats were weighed and monitored daily for ketones (Ketostix, Ames Co., Elkhart, Indiana) and glucose (Testage, Eli Lilly, Indianapolis, Indiana) in the urine with laboratory test strips. Glycosuria was estimated semiquantitatively on a scale from 0 to 4 plus; ketosuria was recorded as zero, small, or large. Protamine zinc (U-40) insulin (Eli Lilly, Indianapolis, Indiana) was administered subcutaneously each morning to maintain 4+ glucose (without ketosis) in the urine. The initial dosage was determined on the basis of body weight and then adjusted according to individual responses to therapy determined by urinalysis. Daily treatments were recorded on treatment report forms. Non-diabetic siblings, BB Wistar rats that did not develop diabetes (BBWnd), and commercially purchased outbred Wistar (W) rats (Harlan Industries, Indianapolis, Indiana) were housed under identical conditions but did not require insulin therapy.

B. Necropsy Procedure

All rats were necropsied as soon as possible following expiration and were stored in a cold room following the discovery of their death or their sacrifice until the time of their necropsy. Each animal was examined externally and
then laid on its back. A midline excision was made from the mandible to the anus and the skin and musculature was reflected to the side. The thorax was then opened and the clavicles were cut. The trachea was ligated above the thyroid and the body organs were removed en bloc. The cranium was opened to remove the brain. All organs were examined individually for gross abnormalities and observations were recorded on Autopsy Report forms. Interesting findings were frequently photographed. Whole body weights and weights of certain organs (heart, lungs, liver, spleen, pancreas, kidney, testes, brain) were routinely recorded. General sources on rat anatomy (Bivin et al., 1979; Hebeland and Stromberg, 1986) were consulted as necessary.

Tissues were fixed in neutral phosphate-buffered formalin, trimmed, placed in labelled cassettes, and then submitted for processing by the histology laboratories in either the Division of Anatomical Pathology or the Division of Neuropathology. Tissue specimens were processed by Autotechnicon (Technicon Corporation, Tarrytown, NY) as follows. Tissues were dehydrated through a graded ethanol series (70%, 80%, 95%, 100%), cleared with xylene, and infiltrated with paraffin. Then, specimens were embedded in paraffin blocks and sectioned at 4-6 microns with a microtome. Sections were floated in a waterbath, mounted on glass slides, and then routinely stained with hematoxylin
and eosin (HE). Tissue and fluid specimens suspected of antemortem bacterial infection were placed in disposable culture collection tubes (Septi-Seal culturette, Marion Scientific Corporation, Rockford, Illinois) and cultured in the Microbiology Division of the Department of Pathology. Specimens were plated on blood agar, chocolate agar, MacConkey's agar, Sabourand agar, thioglycollate broth, Schaedlers blood agar, Schaedlers blood agar with Kanamycine and Vancomycin, Kamanycin Bile esculin agar, chopped meat, and biphasic medium for mycoplasma. Paraffin sections of such tissues were stained with Gram's stain, acid fast blue, or Gomori's methenamine silver for bacteria and fungi.

Histological preparations of the following tissues were routinely prepared and examined (the number in parenthesis indicate the minimal number of tissue sections processed): heart (1), thoracic aorta (1), lungs (2), liver (1), spleen (1), pancreas (2), stomach (glandular and squamous portions) (1), small intestine (1), colon (1), adrenal (2), kidney (2), bladder (1), testes (2), prostate (1), uterus (2 horns, 1 body), ovary (2), mediastinal lymph node (1), mesenteric lymph node (2), brain (2), pituitary (1), and thyroid (3). Parathyroid and thymus were occasionally examined. The skin, mammary gland, muscle, bone, eyes, spinal cord, and bone marrow were histologically examined only when disease was grossly apparent. Histological findings were recorded on Histology Report forms. Photomicrographs were taken with
a Leitz Wetzlar Orthoplan large field microscope equipped with a Leitz Wetzlar Orthomat camera on Kodak Ectachrome 64 film at 50 ASA.

C. Hematology

Rats used in hematological studies were anesthetized with ether, and blood (300 lambda) was collected in capillary whole blood collectors (Microtainers, Becton-Dickinson, Rutherford, New Jersey) by inserting a heparinized capillary tube (American Hospital Supply Corp., Miami, Florida) into the retro-orbital plexus of vessels at the inner canthus of the eye. This technique was chosen because it offers more consistent results than tail vein bleeding (Hulse, 1965). Complete blood counts (CBC), differential white cell counts, and platelet counts were performed on BBWd (16 male and 11 female), BBWnd (12 male and 10 female), and control (9 male and 10 female rats). (Control rats were a Wistar line obtained from Bio Breeding Laboratories in Ottawa, Canada.) CBC's and platelet counts were accomplished with a Coulter S-plus. Platelet counts in excess of 700,000 were diluted 1:2 until there was agreement in two of three simultaneous measurements.

Blood for serum studies was also collected by retro-orbital bleeding. Samples were allowed to clot and then centrifuged in a table top centrifuge (Model HN, International Equipment Co.) with a fixed angle rotor at
1500 rpm. The supernatant was pipetted into polypropylene micro sample tubes (Kew Scientific, Columbus, Ohio) and frozen at -40°C until use. Protein electrophoresis was performed on agarose plates at pH 8.6. Total protein was measured by the biuret method. Samples from five rats in each of the six categories were compared by two-way analysis of variance.

D. DNA Repair Studies

Because eukaryotic cells undergo semiconservative DNA synthesis only during the S phase of the cell cycle, unscheduled DNA synthesis (UDS), a measure of excision repair, can be quantified in non-S phase cultured fibroblasts using an autoradiographic procedure. If S phase cells are cultured with tritiated thymidine and then examined autoradiographically, the nuclei will show intense staining with silver grains while non-S phase cells will not show significant nuclear labelling. However, non-S phase cells undergoing UDS because of repair will contain nuclear silver grains when examined autoradiographically. The number of silver grains per cell undergoing UDS can then be counted and these counts reflect the amount of DNA repair synthesis. In order to measure UDS, hydroxyurea is used to selectively inhibit S-phase DNA synthesis. Hydroxyurea inhibits ribonucleotide diphosphate reductase, an enzyme used to synthesize deoxynucleoside triphosphates from the
corresponding ribonucleoside diphosphates. Hydroxyurea appears to have little or no effect on UDS because UDS does not require a large pool of DNA precursors and can proceed using the pre-existing pool (Friedberg, 1985).

Primary fibroblast cultures were established from skin samples derived from neonatal offspring of either a diabetic BB Wistar rat mating pair or a control Wistar rat mating pair. The pups were killed and their abdomens cleaned with 70% ethanol. Skin samples (1x1 cm) were excised and then washed three times in phosphate buffered saline (PBS). The samples were placed in 0.5 ml of 0.01% trypsin and then minced between two razor blades. The minced dermis, in 25 ml flasks containing 5 ml of 0.01% trypsin, was then digested for 30 minutes in a 37°C water bath/shaker. The contents of the flasks were then poured into 50 cc plastic centrifuge tubes and then inactivated with 40 ml of B medium (Eagles MEM supplemented with 1.5x essential amino acids, 2x non-essential amino acids, and 1.5x vitamins) containing 10% fetal calf serum and 0.02% gentamycin. The tubes were centrifuged at 1100 RPM for 15 minutes and then the medium was decanted. Pellets were resuspended in 8 ml of B₁₀ 2X medium, divided into four T₂₅ flasks (Corning Glass Works, Corning, N.Y.), and incubated at 37°C with 95% air/5% carbon dioxide. The fibroblasts were confluent at 12 days and were harvested with 0.01% trypsin, split 1:2, and plated. The fibroblasts were split 1:2 for each successive passage.
Passage 3 fibroblasts were used for the experiment.

Fibroblasts from each cell line were attached to 22 x 11 mm glass coverslips as described below. The fibroblasts were suspended at a concentration of $9 \times 10^{10}$ cell/ml into 100 mm diameter culture plates containing ten coverslips. Each coverslip received 0.5 ml cell suspension. The fibroblasts were permitted to attach and grow. After two days, the medium was replaced with medium containing $2 \times 10^{-3}$ M hydroxyurea. After 15 hours, the medium was aspirated and the cells washed twice with PBS. For both BBW and W cell lines, the petri dishes were divided into 3 groups: (a) control plates receiving no radiation, (b) plates treated with 20 J/m$^2$ ultraviolet (UV) radiation, and (c) plates treated with 40 J/m$^2$ UV radiation. Next, both the irradiated and unirradiated plates were then exposed to medium containing $2 \times 10^{-3}$ M hydroxyurea and 2 $\mu$Ci/ml tritiated thymidine ($^3$HdThd, specific activity = 20 Ci/mmol, New England Nuclear) for 0, 2, 4, 6, and 8 h. The coverslips were then washed thrice in PBS and then fixed thrice for 5 min in freshly prepared Carnoy's solution. The fibroblast-bearing coverslips were then dehydrated through an ethanol series and mounted on labeled glass slides cell-surface up. The slides were placed in a drying oven. After drying overnight, the glass slides were dipped in a photographic emulsion (0.67% glycerol + 32.67% distilled water + 66.67% Kodak NTB-2) and stored in black slide boxes.
containing calcium carbonate at 4°C. The black slide boxes were tightly sealed with electrical tape. After 6 days, the emulsion coated coverslips were developed with Kodak D19, washed, fixed, and lightly stained with toludine blue. Fibroblast nuclei were viewed with a microscope (630X). Nuclear grains were counted electronically using a Docuval camera and an Artex 880 counter in approximately 50 non-S phase cells on each coverslip. All analyses were performed on grain counts corrected for background. Corrections for background were made by subtracting the mean number of grains present in adjacent equivalent areas without cells on each coverslip. Three coverslips per cell line were examined for most group and dose interactions. The magnitude of scheduled DNA synthesis for each group and dose was equivalent to the average number of grains per nucleus for irradiated cells minus the values for unirradiated cells.

E. Statistics

Statistical analyses were performed as described in individual papers listed in the appendices.
A. Morphology of Organ Systems

1. Pancreas

A wide variety of exocrine pancreatic lesions have been reported in diabetics, but the significance of many of these, if any, remains obscure. Several sources have reported that the diabetic pancreas has a decreased organ weight (LeCompt and Gepts, 1977), but this is minimal in the absence of other significant pancreatic pathology (e.g.- acinar atrophy) since the islets constitute such a small part of the entire organ (Warren et al., 1966). Fibrosis is the most commonly reported finding in the exocrine pancreas of human diabetics and is sometimes associated with a degree of interstitial chronic inflammation. Very high incidences have been reported in some studies (Warren et al., 1966; Kothare, 1974) but the incidence in the control group of non-diabetics may also be nearly as high (Kothare, 1974). Therefore, this is not a specific change seen in the diabetic pancreas. Other frequent findings include fatty infiltration, arteriosclerosis and pancreatitis. Although the incidence of pancreatitis in some studies has been as high as 20-40% (Kothare, 1974), some autopsy studies have
reported low (5%) incidences (Goto et al., 1974; Parson et al., 1968. On the other hand, it is well documented that either acute or chronic pancreatitis may lead to overt diabetes (Johansen and Ornshott; 1972; Warren et al., 1966), but this is a rare mechanism for the onset of diabetes. There is presently no compelling evidence suggesting that pancreatitis is necessarily more common in diabetics than in the general population (Warren et al., 1966).

Rare findings that appear to be somewhat more frequent in diabetics than in the general population are pancreatic carcinoma and pancreatic calculi (Warren et al., 1966). Because of their low incidence, neither warrants further discussion, but I will digress momentarily because of the historical significance of one of these. A case report by Barron in 1920 in which a calculus blocking a pancreatic duct resulted in acinar atrophy with persistence of the islets is credited with suggesting to Banting his classic duct ligation experiment resulting in the discovery of insulin (Bliss, 1982). In summary, exocrine pancreatic lesions may be common in diabetes, but most appear to have limited significance.

On the other hand, the pathology of the endocrine pancreas in diabetes is very important and has been extensively characterized. Furthermore, the islet pathology differs distinctly in IDDM and NIDDM.
In IDDM, the islets show severe and pathogomonic changes (Gepts and LeCompte, 1981). The most striking change is that islets are decreased in both size and number. Frequently, these endocrine cells are scattered as single cells in the exocrine tissue rather than existing as discrete islets. The appearance of pyknotic nuclei and acidophilic cytoplasm in some islets (Warren et al., 1966) lead to the classic description of "atrophic" or "inactive" islets in IDDM. Immunocytochemical studies later showed that essentially only the beta cells were reduced in number and that the diabetic islets were composed predominately of alpha, delta, and PP cells. It has also been determined that beta cells seldom totally disappear in the years following clinical onset of IDDM (Gepts and LeCompte, 1981; Foulis and Stewart, 1984; Rahier et al., 1983; Orci and Perrelet, 1979). In most cases of IDDM, the number of beta cells is greatly reduced at the onset of diabetes, but this is not always the case. Occasionally, hypertrophic islets, composed mainly of beta cells showing signs of functional hyperactivity are seen in the pancreas of young diabetics who die shortly after onset. Another finding that is frequently observed in the islets of IDDM patients that die shortly after clinical onset (usually less than 1 year) is insulitis. Insulitis, a term coined in 1940 by Von Meyenburg, is lymphocytic infiltration selectively involving the pancreatic islets (Gepts, 1977; Foulis and Stewart,
Insulitis usually affects some but not all islets of the pancreas. In most cases, only small lymphocytes are seen, but the presence of macrophages, plasma cells, eosinophils, and neutrophils have also been reported (Wellmann and Volk, 1980). One final lesion that has interesting historical significance is hydropic change, the presence of cells (now known to be beta cells) with clear cytoplasm. In the pre-insulin era, this was a common finding to which great significance was described. It was considered a degenerative change that was forerunner of islet cell atrophy, but this explanation was dismissed in 1951 when Toreson demonstrated that the hydropic change was due to glycogen deposition (Gepts and LeCompte, 1981). Hydropic change is now fairly rare because few cases of diabetes go untreated.

In contrast to the clear pattern in IDDM, the pancreatic islet pathology in NIDDM is extremely variable and is not pathognomonic (Gepts and LeCompte, 1981). Often, there is some decrease in the number of cells in the islets of individuals with NIDDM but this change is not nearly as severe as in IDDM. Others may have beta cell hyperplasia. Probably the most typical lesion in NIDDM is hyalinosis, a change seen in up to 50% of all NIDDM pancreases (Wellmann and Volk, 1980). Hyalinosis is not specific for NIDDM since it is seen on rare occasions in both IDDM and in non-diabetics (Warren et al., 1966; Wellmann and Volk, 1980).
Islet fibrosis is another common finding in NIDDM but it is also occasionally found in IDDM. In summary, none of these changes are specific for (or even usually present in) NIDDM.

Islet pathology is even seen in the infants of diabetic mothers. Several characteristic changes have been reported. These include islet hypertrophy and hyperplasia, an increase in the percentage of beta cells, and eosinophilic infiltrates. These changes have been attributed to either maternal hyperglycemia or insulin antibodies crossing the placental barrier (Barressi et al., 1978).

According to Nakhooda et al. (1978), BBW rat islets typically show three general patterns: (1) normal islet histology in prediabetic and nondiabetic BBW rats; (2) mononuclear cell (occasionally with eosinophils) insulitis at the onset of diabetes and in the following few weeks; and (3) "end-stage" islets, which are small, decreased in number, and composed almost entirely of non-beta cells, and a few granulated beta cells, in chronically diabetic rats. This picture is confirmed by our study and by others (Seemayer et al., 1982a). Most of the BBWd rats in our study had typical small "end-stage" islets (Figure A.7) often with numerous cells with pyknotic nuclei (Figure A.5). These were particularly common in the older BBWd rats and were not seen in BBWnd or W rats. In almost all instances, at least a few small islets remain regardless of the severity of the diabetic state. In several instances,
hyperplastic islets were seen in BBWd rats after the onset of diabetes (Figure A.6). Islet histology in prediabetic and nondiabetic BBW rats was usually normal.

Insulitis was the most common inflammatory pancreatic lesion in both BBWd and BBWnd rats (Table A.1) and is characterized as a mononuclear cell infiltrate (almost always lymphocytic) that is specific for the islets. In most instances, the infiltrate is predominantly peri-insular (Figure A.1) but in the most severe lesions may also include frank invasion of the islets (Figure A.2). Frequently a few eosinophils are present in the periphery of inflamed islets. (Wright et al., 1985; Appendix A).

In BBWd rats, insulitis occurred most frequently in the younger age groups (Table A.1). The ages of BBWd rats with insulitis ranged from 107 days to 317 days of age (mean ±SD=164.1 ± 61.9 days). Since insulitis is transient, it was seen only in those rats that died shortly after onset of hyperglycemia. The longest period of time following onset of diabetes at which a BBWd rat still had full-blown insulitis was 69 days (the mean duration of diabetes for BBWd rats with insulitis when they died was 49.9 ± 16.9 days).

In reviewing the literature concerning insulitis in BBW rats, I was struck by the straightforward and oversimplified picture presented in previous studies. Seemayer et al. (1982a) examined three early diabetic rats
three unstable diabetic rats (7-22 days after detection), three stable diabetic rats (41-63 days after detection), eleven prediabetic rats (four at 50 days and seven at 65 days of age), and six nondiabetic control rats (BBW rats over 120 days of age that had not developed glycosuria). Seemayer et al. (1982a) reported no structural or inflammatory changes in the control (BBWnd) rats and variable degrees of periductal and acinar mononuclear infiltration and insulitis in the prediabetic rats (particularly in the 65 day old group). All three early diabetic rats had insulitis usually involving only the peripheral portion of the islets but occasionally permeating the whole islet. Involved islets were enlarged, edematous, and poorly delineated from the acinar tissue; islet cells showed cytoplasmic swelling, vacuolization, intense acidophilia, refractility, and nuclear pyknosis. Periductal infiltrates were also present. One rat had focal acinar involvement by mononuclear cells. In the three unstable diabetic rats, islets were small, sparse, and showed no evidence of active cellular injury. Rare islets had residual mononuclear cells. Two of the three stable diabetic rats had a single islet infiltrated by limited numbers of mononuclear cells. No islets were normal and several showed evidence of cellular injury. One rat had periductal mononuclear cell infiltrates.
Since insulitis is a transient manifestation of the onset of diabetes in BBW rats, many questions remain to be answered about the time frame surrounding this event. Seemayer et al. (1982a) observed insulitis in most of their 50 and 65 day old prediabetic rats, and they suggest that the destructive process in the islets starts well in advance of the clinical syndrome. It is not presently known whether glycosuria is always preceded by insulitis. If so, why does it remain severe in some BBWd rats 60-70 days after onset of glycosuria and is not seen in others only a few days after onset. Furthermore, why have other investigators observed fullblown insulitis only in early diabetes (i.e. a few days after onset). Our study reveals that it may be quite prominent several months after the onset of glycosuria (Figure A.2). A likely explanation is that our rat populations are different. Seemayer’s study utilized rats that were selected because they were a very homogenous population for each group (i.e. early diabetic, stable, unstable, and prediabetic). In at least some cases, animals within these groups were littermates. This might in part explain the clear pattern in this study. On the other hand, our rats are a rather heterogenous group with respect to both ancestry and age of onset of diabetes. In order to examine whether age of onset had any effect on the persistence of insulitis, we examined all rats that died or were sacrificed within 70 days of onset of glycosuria and
found that 45 BBW rats were in this category, but there was no statistically significant difference in the age at onset of glycosuria for those rats within this subset that had insulitis and those that did not.

Another point of confusion is the 16.3% incidence of insulitis in our BBWnd rats (Table A.1). In the nondiabetic rats, there was no specific age predilection for insulitis as with the diabetic rats. In fact, insulitis was observed in BBWnd rats ranging from 96 to 422 days of age (mean ± S.D. = 306.5 ± 132.4). Only one of these rats could be considered a typical prediabetic rat (i.e. less than 120 days old). If the insulitis is actually responsible for the destruction of beta cells and the subsequent onset of hyperglycemia, then why does insulitis occur in about one-fifth of the BBWnd rats in our study and others. For instance, Nakhooda et al. (1978) also reported an incidence ranging from 17 to 25% in BBWnd rats, although Seemayer et al. (1982a) did not observe insulitis in his BBWnd series. Either these animals were developing overt diabetes at the time of their death (it is well known that many older BBWnd rats eventually convert into BBWd rats) or insulitis does not always result in clinically apparent diabetes.

In addition to insulitis, other inflammatory lesions were also common (Table A.1). Chronic interstitial inflammation (i.e. inflammation with little acinar involvement) was the most frequent of these occurring in all
three groups of rats at incidences between 11% and 14%. Except for chronic interstitial inflammation, no other pancreatic lesions were seen in W rats. Low incidences of chronic pancreatitis (i.e. inflammation with diffuse, extensive acinar involvement), acute pancreatitis, acute and chronic pancreatitis, acute interstitial inflammation, and acute and chronic interstitial inflammation were present in BBW rats. In the acute lesions, eosinophils were often present either exclusively or with neutrophils (Figure A.3). No age pattern was discernable for any of these inflammatory lesions.

Granulomas were occasionally observed in BBW pancreatic tissue (Table A.1). In no instance could any organisms be demonstrated with gram stain, GMS stain, or acid fast blue stain. In several rats, granulomas and insulitis were present together (Figure A.4). Eosinophilic infiltrates were occasionally present with granulomas.

The most common pancreatic lesion in most strains of aging rats is primary acinar atrophy and fibrosis (Berg, 1967; Anver and Cohen, 1979; Burek, 1978). This change is characterized by shrinkage of acini, absence of cell granules, transformation of acini into duct-like structures lined by cuboidal epithelium, and complete degradation of acini with fibrosis of the empty supporting tissue (Berg, 1967). Fat cells are often interspersed within areas of pancreatic lobular atrophy (Anver & Cohen, 1979). One study
has reported asymptomatic "chronic relapsing pancreatitis" to be very common in some inbred rat strains (Kendrey and Roe, 1969). It appears that many of these instances are equivalent to what we refer to as chronic interstitial inflammation rather than actual pancreatitis (i.e. extensive diffuse acinar involvement). They also reported changes consistent with acinar atrophy (always associated with some inflammation) and a few cases of polyarteritis nodosa.

Two types of endocrine pancreatic lesions occur frequently in aging rats: islet atrophy and islet hyperplasia. Islet atrophy is not common and is usually secondary to extensive exocrine damage whereas islet hypertrophy is more common and may occur spontaneously. Beta cells in the latter islets become both hypertrophic and hyperplastic with increased granularity. Intra- and peri-insular fibrosis may become marked and the islets may eventually coalesce to form giant conglomerates or even tumors. This change is usually associated with abnormal glucose tolerance curves.

Neoplastic changes in the pancreas of the rat are exceedingly rare (Altman and Goodman, 1979; Rowlatt, 1967). In fact, a review by Altman and Goodman (1979) states that only one spontaneous exocrine adenoma and 7 spontaneous exocrine adenocarcinomas have been reported in the literature. Islet tumors are more common than exocrine tumors but are still rare. The majority of endocrine
pancreas tumors have been islet cell adenomas. Only seven islet cell carcinomas have been reported in the literature (Altman and Goodman, 1979).

2. Endocrine
a) Thyroid

The relationship between diabetes and thyroid disease is presently uncertain. Published reports pertaining to thyroid function in diabetes are fragmentary and contradictory. Although very little is known about the functional state of the thyroid in diabetes, there is a small subgroup of the human IDDM population for which this relationship is better established. Several studies have reported an association between IDDM and autoimmune thyroid disease (Irvine et al., 1970 and Nerup and Lernmark, 1981). Relevant to this is the observation that HLA-B8 and Dw3 histocompatibility antigens occur with a greater frequency in patients with IDDM, Graves' disease, and Hashimoto's thyroiditis (Christy et al., 1977 and Moens and Farid, 1978). Furthermore, a higher incidence of thyrotoxicosis has been reported in diabetics as well as frequent carbohydrate metabolic problems in patients with thyrotoxicosis. In addition, autopsy studies have reported an occasional association of IDDM and chronic thyroiditis. Other autoimmune disorders in endocrine glands also are occasionally present in human IDDM. This has been termed
"the syndrome of polyendocrine autoimmunity (Bottazzo et al., 1974; Doniach and Bottazzo, 1981) and is now recognized as a clinical entity. The occurrence of a spontaneous lymphocytic thyroiditis in BBWd rats suggests that BBW rats may closely mimic this syndrome of polyendocrine autoimmunity (Sternthal et al., 1981; Wright et al., 1983a, see Appendix B).

In general, non-neoplastic thyroid disorders are infrequently observed in most strains of laboratory rats. Burek (1978) reported occasional stratified squamous-lined ultimobranchial duct cysts, "colloid" cysts, and focal periarteritis of thyroid arteries in several strains of rats. Organized colloid cysts were very frequently observed in all three groups in our series and were occasionally inflamed. Coleman et al. (1977) reported a 0.7% incidence of diffuse thyroid hypertrophy in Fischer 344 male rats. In this present study, hypertrophy was not common and was seen predominantly with severe thyroiditis. Others have reported atrophic changes (Bullock et al., 1968), marked variation in follicular size and colloid content (Anver and Cohen, 1979), and basophilic debris and calcified concretions (Anver and Cohen, 1979) in the follicles of aged rats. Except for cysts, the only lesion seen in the thyroid of any of the W rats was focal C cell hyperplasia in a 551 day old male W rat.
Lymphocytic thyroiditis was observed in 36 BBWd rats (63.2%) and in eight BBWnd rats (42.1%). A complete description of the histology of these lesions, a review of the pertinent literature, and a discussion of the significance of this finding is present in Appendix B (Wright et al., 1983a). During the past decade, spontaneous thyroiditis in BBW rats has been extensively studied and the BBW rat has become an important animal model for experimental thyroiditis (Boitard et al., 1985; Allen et al., 1986a; Allen et al., 1986b; Allen et al., 1987; Allen et al., 1990; Voorby et al., 1990; Gottlieb et al., 1991; Rajatanavin et al., 1991; Allen, 1992).

Although neoplastic lesions of the thyroid are frequently observed in various strains of aged rats, none were seen in our study. Several studies have reported incidences of naturally occurring medullary thyroid carcinomas in 16-40% of several strains of rats including the Long-Evans, Sprague-Dawley, Wistar, WAG/Rij, and wild rat (Rattus norvigicus) (Boorman and Hollander, 1976; Thompson and Hunt, 1963; Gilbert and Gillman, 1958). Medullary carcinomas in the rat arise from C cells; tumors of follicular cell origin are rare in rats (Boorman and Hollander, 1976). Thyroid tumors may also be induced in rats by feeding a low iodine diet (Axelrod and Leblond, 1955). On the other hand, several strains of rats have very low incidence of thyroid neoplasms. Coleman et al. (1977)
reported a 2.1 and 0.7% incidence of medullary thyroid carcinoma and thyroid adenoma respectively in a study on aging Fischer 344 rats.

b) Parathyroid

No relationship between the parathyroid and diabetes has been theorized, but a few scattered reports indicate that the gland may be adversely affected by the diabetic state. Then again, it is likely that every organ in the body is adversely affected. Fraley and Totten (1968) reported a significantly higher (P < 0.05) incidence of histological abnormalities in the parathyroids of diabetic subjects at autopsy. Hansson (1964) reported an increased volume and nuclear size in the parathyroids of alloxan diabetic rats. On the other hand, Warren et al. (1966) reported no consistent abnormality in the parathyroids of diabetic patients, but often found secondary chief cell hyperplasia in patients with diabetic nephropathy.

Although one or more parathyroids were examined in approximately one half of the rats in this study, no parathyroid lesions were observed in any of the three groups of rats. Sporadic non-neoplastic parathyroid lesions reported in other strains of senile rats include interstitial fibrosis, cystic changes, and hyperplasia secondary to renal disease (Burek, 1978; Altman and Goodman, 1979). Some strains of aged rats rarely have parathyroid
lesions and other strains, e.g. Long Evans rats, have incidences as high as 40% (Russfield, 1967).

Parathyroid neoplasms are a very rare finding in most strains of rats (Altman and Goodman, 1979) and these consist almost exclusively of adenomas. Sass et al. (1975) have reported a single parathyroid carcinoma in a 28 month old Fischer 344 rat. This is possibly the only report of a malignant neoplasm of the parathyroid in a rat.

c) Adrenal

The functional state of the adrenal gland in diabetes mellitus is uncertain. Clinical association of diabetes with Cushing's Syndrome (i.e. hyperactivity of the adrenal cortex) is common knowledge. About 25% of patients with Cushing's Syndrome have symptomatic diabetes. Occasional autopsy studies have reported a significantly higher incidence of adrenal abnormalities (Fraley and Totten, 1968) and adenomas (Russi et al., 1945) in diabetics. Irisawa et al. (1966) have reported that the zona fasciculata is characteristically hyperplastic in diabetes. The present knowledge in this area is at best confusing and the true relationship of adrenal pathology and diabetes is difficult to assess.

A few isolated lesions were observed in BBW rats in this study but we have no reason to believe that they are related to diabetes since all are known to occur in
nondiabetic rat strains. Ectopic adrenal tissue (outside the glandular capsule) was observed in two BBWd rats, but this is not an uncommon finding in other strains of rats (Russfield, 1967; Burek, 1978). Another BBWd rat had three distinct adrenal glands. Other isolated findings observed in BBWd rats that have been described in the literature include "foci of cellular alteration" (Burek, 1978), dilatation of sinusoids associated with blood-filled cysts or thrombosis (Burek, 1978; Muraoka et al., 1977; Anver and Cohen, 1979), focal degeneration and necrosis (Burek, 1978; Anver and Cohen, 1979), sudanophilia and pigmentation (Muraoka et al., 1977), hyalinization of the capsule (Anver and Cohen, 1979) and fatty change (Coleman et al., 1977; Cohen et al., 1978). Several types of non-neoplastic medullary lesions have also been described. These changes included "foci of basophilic change" (Burek, 1978) and thrombosis (Cohen et al., 1978). The significance (if any) of non-neoplastic adrenal lesions in rats has not been established; but they may occur frequently in some strains (Burek, 1978).

Although no neoplastic adrenal lesions were observed in this study, spontaneous adrenal tumors are common in some strains of aged rats. Both adenomas and carcinomas are observed in older rats with adenomas predominating (Altman and Goodman, 1979). Incidences of adrenal cortical adenomas between 2-40% have been reported in old rats (Sass et al.,
1975; Cohen et al., 1978; Burek, 1978). Single incidences of myelolipoma and sarcoma have also been reported (Altman and Goodman, 1979). Nothing is known about the endocrine activity of these cortical tumors (Uebeberg and Lützen, 1979). Medullary tumors in rats consist almost exclusively of pheochromocytomas. The incidence and age of onset of this lesion varies drastically even in strains of rats derived from common ancestors. Burek (1978) reported these lesions in only 8% of the females and 1% of the males in Wistar-derived WAG/Rij rats (mean age 34 months) while Gilbert and Gillman (1958) reported a 50% incidence in female and an 82% incidence in male South African Wistar rats living over a year.

d) Pituitary

A relationship between pituitary disease and diabetes has been suspected for many years (Warren et al., 1966). Marie's original paper on acromegaly in 1889 reported that these two diseases may occur together. Harvey Cushing in 1912 produced strong evidence for an association between glycosuria and excess secretion of the pituitary. Experimental observations by Houssay in 1931 lead to the description of the Houssay phenomenon: amelioration of diabetes following removal of the pituitary (Warren et al., 1966). Observations consistent with this phenomenon have since been reported with Sheehan's Syndrome (postpartum
pituitary necrosis), chromophobe adenoma, and late inactive eosinophilic adenoma (Bonar, 1977.) Several investigators have noted the occurrence of the Houssay phenomenon in man due to pituitary infarction, but others have not seen any improvement.

Essentially no consistent morphological changes have been reported in the pituitaries of diabetics in the recent literature, although Warren et al., (1966) did report a three-fold increase in the incidence of pituitary infarcts in diabetics. Autopsy studies on IDDM patients that died in the pre-insulin era showed several characteristic pituitary changes including a reduction in the gross weight of the pituitary, a reduction in the number and size of eosinophils (often with pyknosis), and increased numbers of infarcts and fibrotic foci. Kraus believed these changes to be secondary to IDDM and later suggested that the discrepancies between his study and later studies might be due to the moderating effects of insulin treatment and the inclusion of both IDDM and NIDDM patients in later studies (Kraus, 1944).

Except for a single chromophobe adenoma in a 327 day old male BBWd rat (Figures 4.1 and 4.2), no pituitary lesions were observed in either BBW or W rats in our study. This is undoubtedly an insignificant lesion since tumors of the pituitary gland are extremely common in many strains of aged rats and is the main factor limiting the lifespan of some strains. In general, female rats have a higher
incidence than males (Altman and Goodman, 1979). Incidence is very low in rats under 18 months (Snell, 1963), but adenomas have been reported in rats as young as seven months (Altman and Goodman, 1979). The vast majority of pituitary tumors in rats are chromophobe adenomas. Grossly, most are soft with an irregular surface and have prominent hemorrhagic areas. They are generally well circumscribed and consist of large polygonal cells with prominent vesicular nuclei and abundant non-granulated eosinophilic cytoplasm. Basophilic adenomas, acidophilic adenomas, chromophobe carcinomas, a craniopharyngioma, a granular cell myoblastoma, and a fibrosarcoma have been previously reported in rats but are rare (Altman and Goodman, 1979). Magnusson et al. (1979) has reported a 0.42% incidence of malignant pituitary tumors in outbred Sprague-Dawley rats. These showed infiltration into brain tissue but not metastasized extracranially.

Non-neoplastic lesions of the pituitary are both uncommon and insignificant (Burek, 1978). Colloid cysts are occasionally seen in the anterior pituitary of rats older than 20 months of age. Berg (1967) reports a generalized increase in chromophobe cells and a decrease in acidophils. Increased reticular connective tissue and melanin pigment have also been reported in aged rats (Burek, 1978; Anver and Cohen, 1979).
3. Male Reproductive
   a) Testes

   It is well known that impotence affects approximately 50% of diabetic men of reproductive age (Konez and Balodimos, 1970; Wiles, 1992). Schoffling et al. (1963) attempted to relate testicular histological findings to the clinical presence of impotence because none of the testicular biopsies from diabetic patients with impotence was entirely normal. They found variable degrees of tubular atrophy, basement membrane thickening, decreased spermatogenesis, and a relative preponderance of Sertoli cells. Federlin et al. (1965), also reported a significant decrease in the early stages of spermatogenesis in diabetics with "hypogonadism". Neither Schoffling nor Federlin examined testicular histology in diabetics with normal sexual function. Singhal et al. (1969), examined testicular biopsies from diabetics with and without impotence as well from age-matched normal controls. They reported histological findings similar to the previous studies but also observed that these changes were not limited entirely to diabetics with impotence. Although these changes were more frequent in the diabetics than in the controls, there was no significant correlation with loss of sexual potency. Impotence is presently believed to be due to diabetic peripheral neuropathy and to be unrelated to these testicular changes (Konez and Balodimos, 1970; Wiles, 1992).
Although testicular histology has been studied extensively in chemically-induced diabetes (Scaffidi and Rotolo, 1974; Schöffling et al., 1967; Rosenman et al., 1974; Soulairac et al., 1948; Oksanen, 1975; Lukens, 1948; Chesler and Tislowitz, 1945; Hunt and Bailey, 1961), it has been examined in only two other strains of spontaneously diabetic animals, the obese-hyperglycemic AO mouse (Hellman et al., 1963) and the Chinese hamster (Schöffling et al., 1967). Both of these studies described hypocellularity or maturation arrest as the predominant lesion rather than total absence of germ cells within seminiferous tubules. Histological changes observed in the BB Wistar rat included both mild and severe atrophy, in addition to these milder changes (Wright et al., 1982, Appendix C; Murray et al., 1983; Murray et al, 1985).

Pancreatectomy in the rat also results in degenerative changes of the germinal epithelium (Lema et al., 1965) which were observed only after the onset of diabetes. The degree of change varied with the severity of diabetes. Without exception, the thickness of the basement membrane of the tubules and blood vessels were normal. Atrophy of secondary sexual organs were occasionally observed in rats with severe testicular damage. These investigators also reported tubular changes consistent with maturation arrest and a decreased ability to impregnate female rats. Pancreatectomy has also been reported to result in testicular atrophy in
cats and roosters (Lema et al., 1965).

The severity of testicular lesions in diabetic men and animals varies widely from study to study. Several have reported only a hypocellularity of germinal cells (Hellman et al., 1963; Scaffidi and Rotolo, 1974), others more severe atrophy (Schöffling et al., 1967), and some only Sertoli cells present (Rosenmann et al., 1974; Soulairac et al., 1948). This discrepancy may possibly be explained by the variable dosages and/or toxicities of the chemicals used to induce diabetes or by severity of the diabetic state in these different studies. Several investigators have suggested that testicular atrophy is more frequent in poorly controlled diabetics (Schöffling et al., 1967; Warren and LeCompte, 1952).

Since testicular atrophy is prevalent in most strains of aged rats (Burek, 1978; Coleman et al., 1977; Lutzen and Ueberberg, 1973), age is certainly a contributing factor to the high incidence of testicular lesions in the BB Wistar rats. Burek (1978) reported that at least limited atrophy was present in all rats over 18 months of age and was usually severe in rats older than 24 months. He found that atrophic testes of old rats frequently had Leydig cell hyperplasia, interstitial edema, tubules with multinucleated giant cells or only Sertoli cells. The changes described by Burek were consistent with the histological changes observed in all three types of rats in our study (Wright et al.,
Several investigators have reported benign or malignant tumors to be frequently associated with testicular atrophy in senile rats (Burek, 1978; Coleman et al., 1977; Lutzen and Ueberberg, 1973), but these were not seen in any rats in our study. This is possibly due to the greater age of their animals. In our study, testicular atrophy was first observed in BBWnd and W rats at 355 and 361 days of age respectively; the earliest age of onset in the BBW diabetic rats was 148 days. Figure C.1 shows clearly that the incidence of atrophy increased with age in all rats but occurred at a much younger age in BBWd rats. Saksena et al. (1979) have reported that sperm production in Sprague Dawley rats remains maximal from age 72 days to beyond 450 days of age. This appears consistent with our histological findings in standard Wistar rats but not in the BBWd rats. Age is undoubtedly a very important factor determining the presence of atrophy in all groups of rats but the duration of diabetes is a much better predictor of atrophy in BBWd than is age. Other causes of testicular atrophy in rats are extensively reviewed in Appendix D (Wright, 1987).

Inflammatory testicular lesions were uncommon. Two diabetic rats had orchitis (Figure 4.3) and another had bilateral granulomatous lesions (Figures 4.4 and 4.5).
b) Prostate

Reports of rat prostatic pathology are limited because the organ is often not included in necropsy studies, but the available literature indicates that prostatitis is common (Müntzing et al., 1979). Burek (1978) reported that the most common lesion in the prostate was diffuse, multifocal, or focal lymphocytic and plasmacytic infiltration. Abscesses were occasionally observed. Unfortunately, Burek did not report any frequencies of prostatic lesions. Cohen et al. (1978), reported a 22.9% incidence of chronic prostatitis in aging Cr1:CD(SD) BR rats. This is consistent with the findings of Coleman et al. (1977) who reported a 19.5% incidence in Fischer 344 rats; but they also reported a 33.1% incidence of focal suppurative prostatitis. In this present study, the prostate was examined in only a small percentage of the rats, but it is still apparent that prostatitis is common in all three groups of rats. It is interesting that chronic prostatitis predominated in W rats and acute prostatitis in BBW rats (Figure 4.6). No prostatitis was observed in W rats under one year of age, but chronic prostatitis was seen in three of seven W rats (42.9%) in the 481-600 day old group. No acute prostatitis was observed in W rats. On the other hand, acute prostatitis was observed in one of 15 BBWnd and three of 24 BBWd rats. No age pattern was discerned. In several of these rats the inflammatory infiltrate included many
eosinophils. In one instance, acute prostatitis caused severe bowel obstruction. An isolated case of chronic prostatitis was observed in a 137 day old BBWd rat.

Small eosinophilic or basophilic concretion bodies (similar to corpora amylacea in man) are common in the acinar lumens in the prostates of older rats (Anver and Cohen, 1979). The significance of these concretions is unknown.

Acinar atrophy is apparently an infrequent finding in rats regardless of age. The only report that I could find in the literature was a single case out of 144 male Fischer 344 rats (Coleman et al., 1977). This is consistent with my observations in the Wistar rats (Figures 4.7, 4.8, and 4.9), but the high incidence in the BBW rat is difficult to explain (Figures 4.10 and 4.11). Sufrin and Prutkin (1974) reported that insulin deficiency causes testosterone to have less effect on the sex accessory glands in the male rat. This is consistent with the findings of Hunt and Bailey (1961) who reported prostatic atrophy similar to that observed following castration in untreated alloxan diabetic rats. They observed normal prostatic histology in rats treated properly with insulin. Although diabetes may explain the prostatic atrophy in the BBW diabetic rats, the explanation for the similar findings in their non-diabetic siblings is uncertain. Furthermore, similar findings have not been reported in human diabetics.
Prostatic hyperplasia, consisting of stratification of acinar epithelium, has been occasionally observed in rats greater than two years of age (Anver and Cohen, 1979). It is uncertain whether this is precancerous but Shain et al. (1975) have reported simultaneous incidence of spontaneous adenocarcinomas and prostatic hyperplasia and hypertrophy in the ventral prostate in aged AXC rats. The incidence and types of prostatic tumors have been reviewed by Altman and Goodman (1979).

3. Female Reproductive

a) Uterus

Uterine changes in diabetic women have not been previously reported, except as related to the formation of the placental unit. These changes include excessive placental weight, villous immaturity, villous infarcts, chorangiosis, intervillus thrombi, obliterative endarteritis or thrombosis of fetal stem arteries, thickening of the trophoblastic basement membrane, villus fibrinoid necrosis and fibrosis (Heifetz, 1992; Benirschke and Kaufmann, 1990). Placental morphology in BBW rats has been studied by Brownscheidle and Davis (1981). They reported a slight decrease in mean placental weight and occasional large multilocular cysts in the basal zone, atrophy of the trilaminar trophoblast, and engorgement of the material blood sinuses.
Pathological changes were not seen in the vaginas or uteri of any of the three groups of rats in our study, except for large uterine stromal polyps in a 372 day old BBWd rat (Figure 4.12). Three BBWd and 1 BBWnd rats died while pregnant. Pregnancies were bilateral except in one rat.

Non-neoplastic lesions of the uterus in senile rats are infrequently reported. With advancing age, a gradual transformation of interstitial reticulum into collagen is seen in the uterus, cervix, and vagina. Hydrometra, pyometra, and cystic endometrial hyperplasia have also been reported (Anver and Cohen, 1978; Berg, 1967).

Neoplastic lesions of the uterus are relatively uncommon in most strains of rats. Most uterine tumors are endometrial stromal polyps (Altman and Goodman, 1978), but a wide variety of other benign or malignant tumors have been reported. These have been reviewed by Baba and Von Haam (1967 and 1976).

b) Ovary

Although decreased fertility and pregnancy complications are frequent in female diabetics, ovarian pathology is unremarkable (Warren et al., 1966). Several studies have reported that ovarian atrophy and fibrosis are more frequent in diabetics than in the general population (Fraley and Totten, 1968; Warren et al., 1966) but this is
more likely associated with generalized wasting due to nutritional deficiencies rather than a specific diabetic alteration. This is supported by the observation that the atrophy was more severe prior to the discovery of insulin. In fact, in about two-thirds of autopsy cases prior to insulin therapy the ovaries were less than half the size of those from non-diabetic women (Warren et al., 1966).

No definite lesions were observed in the ovaries of any of the rats in our study. There did appear to be some decrease in the number of corpora lutea in young BBWd rats, but no morphometric studies were done to confirm this. In fact, two BBWd rats under one year of age had no apparent corpora lutea. Ovaries from sexually mature BBWnd and W rats appeared normal.

Ovarian pathology in aging rats has been summarized in detail by Russfield (1967). Basic age-related changes include decreased oocytes, follicles, and corpora lutea and an increase in interstitial gland tissue, cyst formation, and degenerative changes in the corpora lutea. Most of these are features of ovarian atrophy. Burek (1978) claims that most, if not all rats over 18 months of age have atrophic ovaries, but the degree of atrophy is variable. Since there is a strong correlation between abnormal estrous cyclicity and morphological changes in the uterus, ovaries, and mammary gland (Anver and Cohen, 1979), a proper morphologic study must also include vaginal smears to
determine the cycling patterns of the rats. Since breeding problems are common in BBW rats, more definitive studies on this area might be interesting.

Inflammatory changes in the ovaries of female rats are not uncommon and ovarian abscesses are probably the most significant of these (Bullock et al., 1968). On the other hand, ovarian tumors are infrequent in most strains of rats. When present, they are usually granulosa-theca cell tumors. Other types of ovarian tumors are very rare (Altman and Goodman, 1979).

c) Mammary Gland

There are no changes in the mammary glands of diabetic women that are specific to that disease. Merriam and Sommers (1957) observed a frequent presence of hyaline substance surrounding the mammary ducts in diabetic women. The only other pertinent study is one showing that breast cancer is less frequent in diabetic women because its onset occurs much later in life (Anderson, 1971). This may, in part, explain why no mammary tumors were observed in any of the BBWd rats in our study. It is possible that female BBWd rats do not develop mammary tumors because they do not live long enough. The only mammary lesion observed in our study was a single mammary adenocarcinoma in a 248 day old BBWd rat (Figures 4.13 and 4.14). Other histologic abnormalities may have been present but were not diagnosed because only
grossly apparent lesions were examined.

The mammary tumor is the most frequently occurring neoplasm in the female rat (Ueberberg and Lutzen, 1979; Altman and Goodman, 1979). Sher (1972) has reviewed the incidence of these for a variety of strains. Many strains have frequencies around 50% and a few up to 90%. Reports of 15 to 20% frequencies in males are not uncommon (Sass et al., 1975; Altman and Goodman, 1979).

Generally, most murine mammary tumors are fibroadenomas; adenomas are infrequent. Benign and malignant fibromas and other mesenchymal tumors are occasionally seen. Adenocarcinomas occur in less than 10% of all spontaneous mammary tumors. Other carcinomas occasionally seen are papillary carcinomas, comedocarcinomas, and squamous cell carcinomas. Rat mammary carcinomas, while histologically malignant, remain reasonably well circumscribed and only moderately invasive (Altman and Goodman, 1979).

The incidence of mammary tumors increases with age. They are rare before one year of age and the incidence increases rapidly after 18 months (Altman and Goodman, 1979). According to Burek (1978), the incidence of fibroadenomas peaks between 31 and 36 months of age and the risk of occurrence decreases after 37 months. In contrast, he also reported that there is no age where the incidence of adenocarcinomas is higher than any other age. Since the
mammary tissue in the rat extends from the axilla to the inguinal region on either side of the ventral midlines, tumors may present anywhere in this area.

4. Digestive
A) Gastrointestinal

Morphological changes in the gastrointestinal tract are not a characteristic of diabetes mellitus in man, but gastrointestinal motility problems are not infrequent. Decreased motor activities have been demonstrated in the esophagus, stomach, small intestine, and colon (Battle et al., 1980), but these findings are usually present only in diabetics with severe peripheral neuropathy (Battle et al., 1980; Hoeffel et al., 1980). Although gastric retention is seen in a small percentage of diabetics (Hoeffel et al., 1980), constipation is a more common complaint (Battle et al., 1980). There is some evidence that the gastrointestinal complications of diabetes mellitus can be improved with meticulous metabolic control (White et al., 1981).

We observed a low frequency of diabetic rats with severe constipation, distended abdomens, and anorexia. These rats were treated with warm enemas which provided temporary relief but these rats invariably died of this disorder. Several of these were due to bowel obstruction caused by bowel necrosis or prostatitis, but two of these
had no physical obstruction and were termed "idiopathic megacolon" (Figure 4.15). Both of these rats were about one year old and had been diabetic in excess of 200 days. We examined the rectum and colon of these rats with multiple histological sections to rule out aganglionic megacolon. In all instances, the ganglion cells of the myenteric plexus were present. Since the distended abdomen did not appear soon after birth, it was obvious that these ganglion cells were originally functional. Therefore, a possible explanation was that they were involved by autonomic neuropathy (Yagihashi and Simi, 1985a; Yagihashi and Sima, 1985b). Our original finding has since been confirmed by Meehan et al. (1994); they prospectively examined a larger number of cases and found that these lesions also occur in BBWnd rats. This suggests that autonomic neuropathy is not responsible and they have postulated an autoimmune hypothesis (Meehan et al., 1994).

By far the most common lesion in the gastrointestinal tract of BBW rats was gastric erosions and ulcerations (Wright et al., 1981). Gastric erosions were observed in 32.1% of the BBWd and 9.7% of the BBWnd rats. None were seen in W rats. The stomach of the rat has three subdivisions: The forestomach, the fundus and the antrum. The forestomach is lined by keratinized stratified squamous epithelium and is separated from the fundus by a transverse ridge. Both the antrum and the fundus are composed of
glandular epithelium. The former secretes mucus and the latter secretes acid. This anatomical distinction is important when examining gastric erosions and ulcerations. Spontaneous acute ulcerations in the forestomach are a common finding in some strains of rats (Burek, 1978; Berg, 1967; Muraoka et al., 1977), but apparently are not seen in others (Coleman et al., 1977; Berg, 1967). Forestomach lesions may be focal or multifocal and are frequently associated with hemorrhage into the lumen. Histologically, many of these are inflamed and edematous. Some of these may be so severe that the ulcers penetrated the entire stomach wall resulting in peritonitis (Burek, 1978).

Although stress ulcerations of the glandular portions of the stomach may be induced in rats experimentally (Glavin, 1980) they occur spontaneously in rats very rarely, if ever. Except for our report in the BB Wistar rat (Wright et al., 1981, appendix E), I was unable to discover a single reported case in routine rat necropsy studies. Although most investigators fail to mention findings that are absent, Burek (1978) reported that none were observed in his studies. Several of our findings are of interest: (1) glandular erosions (Figures E.1 and E.2) predominated; (2) forestomach erosions (Figures 4.16 and 4.17) were very uncommon; (3) forestomach erosions were always associated with glandular lesions. The morphological and etiological features of these lesions in BBW rats have been described
previously (see Appendix E, Wright et al., 1981).

Interestingly, our study prompted a study by Nishimura et al. (1983) which demonstrated that a high incidence of spontaneous gastric erosions also occur in two other spontaneous models of diabetes, the NOD mouse and the KK-A mouse. In both strains, erosions occurred in both diabetic and non-diabetic mice. No erosions were seen in other mouse strains not predisposed to diabetes that were housed in the same facility. We have no explanation for our findings or those of Nishimura et al. (1983).

Tumors of the gastrointestinal tract are so rare that most reports are single cases (Altman and Goodman, 1979). A total of 22 cases of various types of tumors of the small intestine are in the literature (Altman and Goodman, 1979) and tumors of the colon are equally rare, except for one totally unexplainable short duration epidemic of spontaneous colon adenocarcinomas in AS rats (Heslop, 1969). The near absence of colon cancer in rats is in total contrast to the very high incidence in man (Willis, 1935). The only GI tract neoplasms observed in our study were lymphomatous infiltrates present in the colon and distal small bowel of a few of the BBW rats. These will be discussed in the section on the lymphoid system.

With the exception of the hemorrhaging ulcers, it is very questionable whether any of these GI tract lesions are very significant, life-threatening maintenance problems.
The relationship between liver disease and diabetes mellitus has been extensively studied, but definite answers are lacking. It is now believed that in some cases liver disease may be induced by diabetes; in others, diabetes by liver disease (i.e. Naunyn's "liver diabetes"); and in still others, both diseases may result from a common disorder (Creutzfeldt et al., 1970). Because of the liver's important role in carbohydrate metabolism, liver diseases would seem a natural consequence of diabetes mellitus, but this has been difficult to prove. In other words, there does not appear to be a specific diabetic "hepatopathy" as in the case with diabetic nephropathy, or retinopathy, or neuropathy. Recently, a lesion called "diabetic hepatitis" has been described in a subset of type II diabetics (Nagore and Scheur, 1988). This lesion resembles alcoholic hepatitis. The constant findings are fatty change, glycogen vacuolization of hepatocyte nuclei, and hepatocyte ballooning. Inflammation, acute or chronic, is variably present as are Mallory bodies and fibrosis. This trend is also seen in diabetic children (Lorenz and Barenwald, 1979).

A high frequency of gallstones has been reported in diabetics at autopsy, but it is now apparent that this is not true (Creutzfeldt et al., 1970). The simultaneous occurrence of diabetes and cholelithiasis can be explained simply on the basis of common obesity.
The relationship between diabetes and steatosis is difficult to assess (Creutzfeldt et al., 1970; Stone and van Thiel, 1985). Liver biopsy studies and autopsy studies have reported incidences of 21%-78% and 20%-30% respectively. The true incidence of fatty liver in diabetes is unknown because liver biopsies are performed selectively on those with hepatomegaly or abnormal bromsulphalein retention and also because autopsy statistics are not representative due to the complications of the terminal diseases. Furthermore, the true incidence of fatty liver in the "normal" population is difficult to estimate, since these people are unlikely to be biopsied. These studies are further complicated by the obesity factor, since approximately 50% of obese persons have fatty livers. It is therefore difficult to conclude that steatosis in obese diabetics is a result of diabetes (Creutzfeldt et al., 1970). Still, a significant, positive correlation exists between age of onset and fatty liver. In IDDM, the incidence of fatty degeneration is low and appears to be controlled with insulin treatment. No such correlation with good blood sugar control was observable in type II diabetics (Creutzfeldt et al., 1970). The fatty change is thought to be due to an imbalance between the level of triglyceride synthesis and the ability of the liver to secrete triglycerides in VLDL particles (Stone and van Thiel, 1985).
Lipid overloaded Kupffer cells appear to be common in diabetes, but, once again, this condition is not specific for diabetes. In these instances the whole reticulo-endothelial system may be likewise involved (Cabarrou et al., 1973).

The incidence of liver cirrhosis appears to be significantly elevated in diabetes mellitus (Creutzfeldt et al., 1970; Stone and van Thiel, 1985). Autopsy studies have cited this incidence to be between 5.7% and 21.4% with male diabetics more frequently afflicted than females. Clinical studies indicate that the onset of diabetes is frequently preceded by cirrhosis. It is, therefore, unlikely that diabetes has a causative role in the pathogenesis of cirrhosis, and the nature of this relationship is presently unknown.

The diabetic liver is rich in glycogen, but the amount of hepatocyte glycogen does not normally correlate with fasting blood glucose, the type of diabetes, the degree of ketosis, or the fat content of the cells. Nuclear glycogen deposits are found in 60-75% of the diabetic population, but this change is not specific for diabetes mellitus (Chips and Duff, 1942; Creutzfeldt et al., 1970). There is some evidence of a reciprocal relationship between cytoplasmic and nuclear glycogen in diabetes, but this is debated. Although both mode of development and the significance of nuclear glycogen is presently unknown, nuclear glycogen does
not occur in normal subjects.

The incidence and types of hepatic lesions in rats vary greatly depending on the age and strain (Anver and Cohen, 1979). Because of the wide-spread utilization of rats in drug and chemical studies, the naturally-occurring hepatic pathology of rats has been extremely well-characterized. In fact, a workshop was held to standardize the classification of specific hepatocellular lesions in rats (Squire and Levitt, 1975). Cholangiofibrosis, mucopolysaccharide-containing cysts, and telangiectasis are the most frequently observed age-associated non-neoplastic hepatic lesions (Anver and Cohen, 1979).

Non-neoplastic hepatic lesions were relatively uncommon in our study (Table 4.1) and presented a largely random, non-strain related pattern. Hepatic fatty change, although uncommon, was somewhat more frequent in BBWd rats than in BBWnd and W rats (Figure 4.18). Since the BBWd rat is a non-obese model for IDDM, a low incidence of fatty change is consistent with findings in human diabetics. Mild periportal inflammation (data not shown) and hepatic infarcts were occasionally present in all three groups of rats. The latter finding is surprising in light of the dual blood supply of the liver. Other isolated findings included an abscess, bile duct hyperplasia, and centrilobular necrosis. Similar findings have since been reported by Bernuau et al. (1985). These investigators also performed
morphometric studies demonstrating perisinusoidal fibrosis that was strain-specific but not diabetes-specific.

Probably the most interesting hepatic lesion in this study was a rare developmental abnormality, a supradiaphragmatic accessory lobe of the liver (Wright et al., 1983b, appendix F), that has been reported in only one other strain, the Gunn rat (Machado and Lozzio, 1972). Supradiaphragmatic accessory livers were present in two very closely related BBW rats, one a diabetic male and the other a nondiabetic female. Since this abnormality has been found in only two strains of inbred rats, the pattern of occurrence suggests a genetic mode of inheritance that is either polygeneic or autosomal recessive with low penetrance. The gross (Figure F.1) and histologic (Figures F.2, F.3, and F.4) appearances of these lesions are shown in appendix F (Wright et al., 1983b).

No hepatic neoplasms were observed in this study. Evaluation of the literature pertaining to hepatic neoplasms in rats is difficult due to past confusion and ambiguous terminology. The term "hepatoma" does not indicate whether the tumor is benign or malignant. Some sources believe that benign hepatic tumors do not exist in rats (Squire and Levitt, 1975). Another frequently used confusing term is the "hyperplastic nodule", a discrete circumscribed nodule of proliferating hepatocytes which compress the surrounding parenchyma. These are believed to be preneoplastic.
Spontaneous hepatic sarcomas have frequently been reported in the past, but these are now believed to occur only in rat colonies infected with *taenia taeniformis*, the cat tapeworm (Altman and Goodman, 1979). This parasite is known to induce hepatic sarcomas in rats. Therefore, sarcomas in rats should not be considered spontaneous. On the other hand, hepatocellular carcinomas do occur spontaneously with a low incidence.

6. Urinary
a) Bladder

Urinary bladder infections and bacteriuria are common in diabetes, particularly in diabetic women. The prevalence of bacteriuria in diabetic women has been reported to be 11-18%. This is approximately two to three times the rate in nondiabetic women (Schoenbaum, 1979). Although a number of factors are involved, the occurrence of urinary bladder infections appears to correlate best with the degree of microangiopathy. Other risk factors included neurogenic bladder, prior instrumentation of the urinary tract, and duration of diabetes for greater than 20 years (Schoenbaum, 1979). Warren et al. (1966), reported "readily recognized" cystitis in 52 or 351 diabetic autopsies.

Urinary bladder pathology was rarely seen in our study. Only one rat, a female BBWnd rat of unknown age, developed cystitis. Calculi of the bladder were infrequently seen in
BBW rats and did not result in infection. Urolithiasis has been reported to be common in several strains of rats and to be rare in others. These may be found in the renal pelvis, ureters, or bladder (Magnusson and Ramsay, 1971; Anver and Cohen, 1979) and can cause stasis, dilation and infection. Other non-neoplastic lesions of the bladder appear to be uncommon in rats. Neoplastic lesions are also rare and have been reviewed by Altman and Goodman (1979).

b) Kidney

One of the most severe clinical sequelae of IDDM is diabetic nephropathy. In fact, renal disease accounts for the deaths of approximately one half of all diabetics that die while under the age of 20 (Westberg, 1980). Presently, diabetic glomerulopathy is the leading cause of renal insufficiency in the United States (Stout et al., 1994). A multitude of different forms of renal disease are more frequent in diabetics than in the general population, but most of these are infections that are secondary to involvement of the bladder by autonomic neuropathy. Diabetic glomerulosclerosis is the only specific form of renal disease seen in IDDM. Not only is it specific for diabetes, its incidence increases with the severity of the carbohydrate intolerance. Diabetic glomerulosclerosis is by far the most important form of renal disease seen in diabetic patients.
There are essentially two basic changes present in all forms of diabetic glomerulosclerosis, basement membrane (BM) thickening and mesangial thickening (Mauer et al., 1981). There is considerable evidence that both of these changes may be due to non-enzymatic glycosylation (Brownlee, 1992).

Osterby (1975) has shown by morphometric analysis of electron micrographs that both BM thickening and mesangial thickening are absent in young patients shortly after onset of IDDM, but are apparent within two years and are prominent within five years of onset. Morphologically, diabetic glomerulosclerosis may take two distinct courses, benign or accelerated. The benign course consists of concurrent thickening of the capillary basement membrane and diffuse glomerulosclerosis (i.e. - mesangial thickening without nodules). This may progress slowly over many years but rarely leads to renal failure. There is also an accelerated course which is superimposed on the changes seen in the benign course. This is a rapid process that frequently leads to renal failure (Bloodworth, 1978).

The natural history of the accelerated lesions has been extensively studied by Bloodworth (1978). First, the changes seen in benign diabetic glomerulosclerosis (i.e. capillary BM and mesangial thickening) occur. After 5-10 years of diabetes (regardless of age), renal arterio- and arteriolar sclerosis are present with some resulting ischemia. Glomerular capillary microaneurysms, sometimes
occupying as much as one-third of the diameter of a
glomerular tuft, develop. The mesangium of the lobule from
which the microaneurysm originated proliferates rapidly and
pushes laterally into the microaneurysm forming a nodule.
These nodules are originally quite cellular and contain fine
fibers and osmiophilic granules between cells but they
eventually develop into hyalinized masses (Kimmelstiel-
Wilson nodules) that are negative to amyloid stains and
weakly positive with eosin and PAS. Silver stains
frequently show a laminated pattern. Kimmelstiel-Wilson
nodules are considered pathognomonic for diabetes but are
found in only 9-25% of diabetic patients. Large
microaneurysms of Kimmelstiel-Wilson nodules are often found
adjacent to glomerulo-capsular adhesions, areas of fibrosis
binding the peripheral glomerular capillaries to the
adjacent parietal layer of Bowman’s capsule. Usually both
parietal and visceral epithelium are absent in these
adhesions. Bloodworth suggests that these are due to large
structures (e.g. Kimmelsteil-Wilson nodules) pushing the
glomerular tuft against the capsule. Although few diabetic
kidneys reach this stage, obliterative diabetic
glomerulosclerosis is the endstage lesion in this process
and is believed to be a result of ischemia due to a
combination of pressure from excessive deposition of BM and
due to arterio- and arteriolar sclerosis of renal vessels.
These glomeruli eventually degenerate to a compact tangle of
BM material. This has been described as the "large hyaline ball" change.

Diabetes is also characterized by a series of insudative lesions (i.e., intramural accumulations of plasma proteins and lipids) which can be subdivided by location (Stout et al., 1994). Insudative lesions within Bowman's capsule are called capsular drops. Within peripheral glomerular capillaries, insudative lesions are called fibrin caps. Stout et al. also suggest that insudation within afferent and efferent arterioles is responsible for the hyalinization of these structures that is common in diabetics. However, none of these lesions is 100% specific for diabetes (Stout et al., 1994).

Inflammatory lesions are also frequent in the diabetic kidney. In the later stages of diabetes, autonomic peripheral neuropathy often leads to bladder dysfunction and urinary stasis. This may result in urinary tract infections. Acute febrile pyelonephritis and glomerulonephritis are common complications of diabetes. Therefore, when a diabetic develops signs of renal disease earlier than expected, some type of kidney infection should be suspected (Westberg, 1980). Surprisingly, there is little evidence that one form of renal infection, chronic interstitial nephritis, has a higher incidence in diabetics (Westberg, 1980).
Renal lesions were very uncommon in our study. Cysts containing proteinaceous fluid were occasionally seen, but these were non-specific findings and were not tabulated. Infrequently, the formation of these cysts from dilated tubules was observed to be associated with focal lymphocytic inflammation (Figure 4.19) and acute pyelonephritis. According to Burek (1978), this is typical of the chronic progressive glomerulonephropathy characteristic of the rat.

Chronic renal disease is a major cause of morbidity and mortality in many strains of rats (Anver and Cohen, 1979). These changes appear to be unrelated to infection because chronic renal disease is also seen in specific pathogen free and germ free rats (Anver and Cohen, 1979). In some strains, this appears to be related to nutritional factors (Bras and Ross, 1964; Snell, 1967; Anver and Cohen, 1979).

The morphology of chronic renal disease in rats is well established (Anver and Cohen, 1979; Berg, 1967; Hirokawa, 1975). The initial lesion occurs at 3-6 months of age and consists of an eosinophilic PAS-positive thickening of the glomerular mesangial matrix. At first, only scattered glomeruli are affected. End stage changes may be seen in rats as young as one year old. Glomerular changes include basement membrane thickening in the capillary tufts and Bowman's capsule, eosinophilic deposits, adhesions between the parietal and visceral layers of Bowman's capsule, and partial or complete hyalinization or sclerosis. Many of the
tubules are dilated or cystic with flattened epithelium and proteinaceous casts in the lumens while others are collapsed and atrophic. The cortical interstitium is fibrotic and infiltrated by mononuclear cells. Protein excretion increases from 5 mg/dl in young rats to 20 mg/dl in most rats over 2 years of age.

Immunofluorescence studies have shown that immunoglobulins are present in the glomeruli. Couser and Stilmant (1976) reported that IgM was bound in the mesangium of 50% of glomeruli of 3-6 month old Sprague-Dawley rats. IgG deposits were minimal. Hirokawa (1975) reported a progressive increase with age in IgG deposition in the glomerular basement membrane and mesangium. Circulating antibodies to renal or other tissue antigens are not present in serum from aged rats with extensive glomerulosclerosis (Couser and Stilmant, 1976). Both authors agree that glomerulosclerosis in rats is not an age-related autoimmune phenomenon and that antigens within the immune complex are probably exogenous in origin.

Inflammatory lesions were also uncommon in our study. Small focal granulomas, present in only five BBWd rats, were the most common inflammatory finding in the kidney (Figure 4.20). Incidental findings included chronic interstitial nephritis (Figure 4.21) in one BBWd and focal acute pyelonephritis in two BBWnd rats.
Although, nephroblastomas, adenomas, adenocarcinomas, mixed tumors, lipomas, sarcomas and hamartomas have been reported in rats, no renal neoplasms were seen in our study. Nephroblastomas are the most common, but renal neoplasms are rare in most strains of rats (Altman and Goodman, 1979).

Although the kidneys of rat and man are embryologically and histologically very similar, they are physiologically very different (Snell, 1967). For instance, man may rely on sweat glands to remove a significant percentage of his body fluid; rats have no such glands. Furthermore, the concentrating ability of the rat kidney far exceeds that of man. Man frequently produces renal amyloid, but this is not found in the rat (Snell, 1967). Therefore, it seems a bit tenuous to expect great similarity in the patterns of human and rat renal pathology. This appears to be the case, since no lesions resembling human diabetic nephropathy were seen in BBWd rats. It is also possible that none of the rats in this study lived long enough to develop renal manifestations of diabetes.

7. Respiratory (Lungs)

One of the very early historical observations in the study of diabetes was that diabetics were more susceptible to pulmonary infection. In fact, one older study reported a 50% positive rate for tuberculosis in autopsied diabetics (Root, 1934). Statistical studies of past influenza
epidemics with high mortality indicate that diabetics are at a higher risk of complications and death following influenza infection (Schoenbaum, 1979). Other types of pulmonary infections are also frequently seen in diabetics. Pneumonia has been recently reported as the second leading infectious cause for hospitalization of diabetic patients (Whitehouse, 1973). On first glance, this appears to be particularly interesting in light of the high incidence of bronchopneumonia in the BB Wistar diabetic rats, but only if one fails to remember that the BBW nondiabetic siblings also have a similar incidence of bronchopneumonia (Table 4.2). It is also important to consider the absence of bronchopneumonia in any of the standard Wistar rats. Since all animals were housed in the same room under similar conditions, one would expect a similar incidence of bronchopneumonia in all three groups. Since this was not observed, it is obvious that the BBW strain is more susceptible to pulmonary infection and that this is unrelated to the diabetic state. This observation, along with others that are mentioned elsewhere in the text, suggest that some form of immunosuppression may play an etiological role in the many afflictions of the BB Wistar rat.

It has been suggested that most laboratory animals have an organ system that is so frequently diseased that it seriously restricts the usefulness of the species for
research purposes. In the rat this is the respiratory system and the disorder is murine chronic respiratory disease (CRD) (Lindsey et al., 1971). Murine CRD is a serious, contagious syndrome that is ubiquitous. A British study reported a high incidence of CRD in commercially available specific pathogen free rats which were cesarean derived and maintained under strict isolation (Lamb, 1975). Lamb histologically examined the lungs of 200 rats from nine major breeders and found that only two of the nine sources had rats with consistently normal lungs (Lamb, 1975).

The natural history of CRD in the rat is reasonably well defined. Newborn rats are almost always normal until weaning (approximately three weeks). A few of the animals in an affected colony show minimal encrustations around the external nares and eyes by 4-8 weeks. These animals make abnormal "snuffling" sounds which are indicative of catarrhal rhinitis. At the same time, many animals develop a suppurative otitis media that is clinically inapparent. Some of these animals develop lesions characterized by varying degrees of peribronchial infiltration of lymphoid cells and bronchiectasis with squamous metaplasia of the bronchial epithelium due to mucous plugs. This stage is the most severe seen in W rats in our study. BBW rats often had more severe disease with one or more lobes containing massive multifocal abscesses (Figure 4.22). Mortality rate for CRD is usually low in younger rats, but is much higher
in rats over 2 years old. The incidence of CRD may be as high as 50 to 100% (Lindsey et al., 1971).

Rats from all three groups (BBWd, BBWnd, and W) had some degree of respiratory disease. The diagnosis of bronchopneumonia was made only when neutrophils could be demonstrated within alveoli (Figure 4.23). When neutrophils were seen only in bronchi, acute bronchitis was diagnosed. Since CRD is ubiquitous and, therefore, essentially "normal" in the rat, this diagnosis was not tabulated, but Figure 4.24 shows one of the most severe cases seen in a W rat in this study. Since CRD may progress to pneumonia (Lindsey et al., 1971), it seems likely that the bronchopneumonia in the BBW rats is simply a more severe reaction of an immuno-compromised strain to microorganism in their environment in spite of the semi-barrier housing facilities. The etiology of CRD is uncertain and the following agents have been suggested: bacteria, conventional viruses, slow virus, Mycoplasma pulmonis, and various combinations of these agents. At present, the case for M. pulmonis is strongest (Lindsey et al., 1971).

Lung specimens from rats with grossly apparent pneumonia were sent to the Microbiology Division of the Pathology Department of University Hospital for examination for bacteria, fungus, and/or mycoplasma. The results of these examinations were most frequently negative or believed to be due to postmortem growth. A complete listing of
microbiological findings is included in Table 4.3. Although Mycoplasma was never demonstrated, there is strong circumstantial evidence implicating this is in the pulmonary lesions in the BB Wistar rat. First, tetracycline is effective in suppressing these symptoms in the BBW rat as has been reported in standard rats experimentally infected with Mycoplasma pulmonis (Lindsey et al., 1971). Secondly, the location of pulmonary lesions following gross examination is similar in both of these groups. The anterior lobe of the left lung and the cranial and azygous lobes of the right lung were the most frequent sites of infection.

Small granulomatous lesions were seen in two BBW rats (Table 4.2), but both were negative for organisms. In addition to these, multifocal histiocytosis was infrequently observed in the lungs of BBW rats. Grossly, these appeared as small brownish discrete or confluent nodules. Histologically, the lesions consisted of focal accumulations of foamy macrophages in expanded alveolar spaces. These findings are of limited significance and are frequently observed in other strains of aged laboratory rats (Yang et al., 1966).

In summary, BBW rats are very susceptible to a variety of pulmonary disorders, the most severe of which is bronchopneumonia. These infections may occur in epidemics and could exterminate whole colonies of rats if extreme care
is not exercised.

8. Circulatory
   a) Heart

   In affluent societies, almost 50% of diabetics die of heart disease (Asmal et al., 1980; Pyörälä, 1989). The risk of myocardial infarction is estimated to be 2 1/2 times greater than that of non-diabetics (Asmal et al., 1980) and to be even higher in obese female diabetics (Sinclair-Smith, 1979). The Framingham study found that diabetic men have twice the frequency of congestive heart disease as non-diabetic men and that diabetic women have a five-fold increased risk (Kannel et al., 1974). The International Atherosclerosis Project, an autopsy study of coronary arteries from over a dozen countries, also found a marked increase in the extent to atherosclerotic lesions in diabetics compared to control patients (Pyörälä, 1989). Although there was a slight increase in fatty streaks, the increase in raised lesions was much more dramatic.

   Until relatively recently, the increased incidence of heart disease in diabetics was considered to be entirely a manifestation of accelerated atherogenesis, but this does not seem to be the case. Several studies utilizing non-invasive techniques for studying left ventricular function have shown a characteristic pattern of shortened left ventricular ejection time and a prolonged pre-ejection
period in diabetic patients. Some of these patients had normal coronary arteriograms. These studies and others suggest that the heart muscle is abnormally stiff in diabetics (Ledet et al., 1979). Similar results have been obtained using alloxan diabetic dogs (Regan et al., 1974). Furthermore, histological studies have suggested that microangiopathy alone is not responsible for diabetic heart disease (Sinclair-Smith, 1979). One study (Ledet, 1976) reported only minor endothelial proliferation and increased PAS material in arterioles, but no changes in capillaries.

Present evidence suggests that diabetic cardiomyopathy is a multifactorial disorder. Vascular disease is probably a major factor, but other biochemical and metabolic abnormalities are also important (Sinclair-Smith, 1979; Ledet et al., 1979).

Most cardiac lesions in rats are age-associated (Wilens and Sproul, 1938). In fact, heart lesions are seldom seen in rats under 12 months of age but are common in rats older than 18 months. The most frequent lesion is myocardial degeneration with fibrosis. This lesion is not usually observable grossly, but may be quite extensive in histological sections. The microscopic appearance is myocardial atrophy, degeneration, and necrosis with condensation fibrosis of the stroma and an inflammatory infiltrate consisting of lymphocytes, Antischkow cells, and macrophages (Figure 4.25). The most frequent site of
involvement is the papillary muscles and their attachment sites in the wall of the left ventricle (Burek, 1976; Anver and Cohen, 1979). Maximum incidence may be as high as 60–80%. Cardiac performance is not impaired (Anver and Cohen, 1979). Table 4.4 shows that low incidences of these lesions were observed in the older age groups in all three groups of rats in our study.

Endocardial and subendocardial proliferative lesions have also been reported in several strains of rats including Wistar rats (Anver and Cohen, 1979). This condition is most common in rats between 25 and 30 months of age. Early lesions consist of subendothelial, undifferentiated, mesenchymal cells and scattered lymphocytes. Advanced lesions had subendocardial accumulations of fibroblasts and collagen. The left ventricle is most commonly affected (Boorman et al., 1973). One lesion of this type was observed in a 198 day old female BBWd rat which also had a lymphoma. Other lesions occasionally found in the hearts of aged rats include intracardiac thrombi and valvular endocarditis (Anver and Cohen, 1979).

Acute myocarditis was observed in two BBW rats. One was in response to a focal fungal infection at the site of an indwelling catheter. The etiology of the other acute myocarditis is not known.
b) Vasculature

Atherosclerosis is presently responsible for the death of approximately three-quarters of American diabetics, but accounted for only 16-23% of diabetic deaths in the pre-insulin era (Warren et al., 1966). Although some of this difference can be explained by the former tendency of insulin-dependent diabetics to die of ketoacidosis and emaciation, the vast majority of diabetics, then and now, are non-insulin-dependent and, therefore, not susceptible to ketoacidosis. Therefore, other explanations obviously must be invoked.

Approximately 75% of diabetics less than 40 years of age have clinically significant atherosclerosis (<5% in non-diabetic controls) and essentially all diabetics, those with IDDM or NIDDM, have clinically significant atherosclerosis within ten years of onset (Bierman, 1992).

The mechanism(s) by which diabetes mellitus influences atherogenesis is not fully understood (Bierman, 1992; Schwartz et al., 1992; Ross and Agins, 1992). It is well established that platelet aggregation is enhanced in diabetes. In addition to clotting abnormalities, evidence suggests that diabetic serum contains elevated levels of various factors (e.g. - growth hormone, insulin) that enhance the growth of arterial smooth muscle cells. It is well-known that non-insulin-dependent diabetics may secrete more insulin as a result of their insulin resistance, even
in the absence of obesity. This is particularly interesting in light of the evidence suggesting that hyperinsulinemia is a risk factor in diabetic atherogenesis. Other factors tending to increase the incidence of atherosclerosis in type II diabetics include obesity, hypertension and hyperlipidemia. Finally, non-enzymatic glycosylation of low density lipoproteins (LDL) appears to promote its affinity to LDL receptors while non-enzymatic glycosylation of high density lipoproteins (HDL) promotes its degradation. Therefore, the athero-protective HDL is lost while the effects of athero-promoting LDL is enhanced. All of these factors combine to promote atherogenesis in diabetes (Bierman, 1992; Schwartz et al., 1992).

According to Warren et al. (1966), the most frequent sites of fatal atherosclerotic lesions in diabetics are the heart (78.7%), brain (10.6%), kidneys (6.4%), and extremities (3.2%). When these values were compared to their own nondiabetic autopsy series, only the prevalence of lethal cardiac and peripheral artery atheromas are significantly increased. They also noted a tendency in diabetics for more severe involvement of muscular arteries rather than the larger elastic arteries.

Several roentgenographic studies have observed that Monckeberg's sclerosis (medial calcification) is more common in diabetics. Ferrier (1964) observed two to three-fold increases in the prevalence of medial arterial calcification
in the knees (20.4%) and feet (23.6%) of diabetics when compared to nondiabetics. Medial calcification also occurs at a much younger age in diabetics. In fact, one study reported an 18.3% incidence of medial calcification of leg arteries in diabetic children (Warren et al., 1966). Fortunately, medial calcification is rarely associated with any clinical consequences.

Diabetic microangiopathy is another devastating vascular complication of diabetes (Laffel and Krolewski, 1989). It is characterized by diffuse basement membrane (BM) thickening. It involves capillaries in most organs but may be most symptomatic when skin, retina, or renal glomeruli are significantly involved. It may also involve non-vascular structures such as renal tubules, Bowman's capsule, peripheral nerves, seminiferous tubules, and placenta villi. The BM thickening can be best demonstrated at the light microscopic level as a hyaline-like material after periodic acid-Schiff (PAS) staining. Even though the BM is thickened, the involved capillaries tend to leak plasma proteins. Advanced glycosylation end-products play an important role in the pathogenesis of microangiopathy (Brownlee, 1992).

Unfortunately, no atheromatous lesions were observed in the thoracic aorta, coronary arteries, cerebral arteries or any other visceral arteries of any of the rats in our study. This is not surprising since atherosclerosis has been only
infrequently observed in autopsy studies on senile rats. This is presumably, in part, because HDL, which has antiatherogenic properties, is the major lipoprotein fraction in the rat, whereas LDL, which is atherogenic, predominates in man.

Several investigators have extensively studied atherosclerosis in the rat. Wilens and Sproul (1938) observed medial calcification and necrosis in the aorta, iliac, coronary, renal, cerebral, and other visceral arteries of Mendel-Sherman rats. Berg (1967) has also occasionally found medial calcification in the aorta and iliac arteries of senile rats. Anver and Cohen (1978) suggest that aortic lesions in rats usually resemble Monckeberg's medial sclerosis rather than atherosclerosis. On the other hand, coronary atherosclerosis, with both medial and intimal lesions, has been reported in various strains of aged rats that have been repeatedly bred (Wexler, 1964). Medial lesions include smooth muscle edema and hypertrophy (sometimes causing lumen stenosis), basophilic degeneration, and mucopolysaccharide vacuolization; intimal lesions are internal elastic membrane disruption, endothelial hyperplasia, and subintimal mucopolysaccharide accumulations (Wexler, 1964). Carotid and cerebral arteriosclerosis has also been reported in senile, repeatedly bred Sprague-Dawley rats (Wexler and True, 1963). One interesting observation that resulted from the preceding
studies is that islet hyperplasia (Wexler and Fischer, 1963a) and glucose intolerance (Wexler and Fischer, 1963b) frequently accompany arteriosclerosis in breeder rats.

The most common vascular pathology seen in senile rats is polyarteritis nodosa (PAN). Frequencies between zero and 60% have been reported in various rat strains (Berg, 1967). The incidence in Wistar rats is reported to be extremely low (Anver and Cohen, 1978). PAN is a vasculitis of unknown etiology that involves muscular arteries. A single artery may have lesions varying from acute to chronic. Prominent histologic features are degeneration of the media and adventitia, fibrinoid necrosis, marked infiltration of PMNs and mononuclear cells, and disruption of the internal elastic lamina. Pancreatic, mesenteric, and spermatic arteries are most frequently involved (Anver and Cohen, 1978). Acute vasculitis and/or polyarteritis nodosa were seen infrequently in BBWd and BBWnd rats (Figures 4.26, 4.27, and 4.28), but were never seen in W rats. In one pregnant rat this was also associated with disseminated intravascular coagulation (Figure 4.29).

Although morphometric studies were not performed, the walls of the muscular arteries in many of the BBW rats appeared thicker than the walls of arteries with similar lumen size in W rats. This may suggest that BBW rats are somewhat hypertensive.
No lesion suggestive of microangiopathy was seen in any of the rats. PAS staining for basement membrane was not routinely performed.

9. Hematopoietic/Lymphatic
a) Thymus

Since the insulitis associated with the onset of IDDM is probably a cell-mediated immune response, it is likely that the thymus is involved in the initiation of this process. In fact, Like et al., (1982) has reported that neither insulitis nor diabetes develops in BBW rats that have had neonatal thymectomies. Nonetheless, thymic lesions are not reported in autopsy studies of juvenile onset diabetics. I was able to find only one study specifically examining the thymus. Souadjian et al. (1970) reported a significant increase in the number of Hassalls' corpuscles and in the diameter of the largest Hassall's corpuscle as well as a decrease in the number of epithelial cells per unit area when comparing the thymuses of juvenile-onset diabetics to those of age-matched controls. The significance of this finding is unknown but it might be more rewarding to examine thymic function rather than morphology in such cases.

Atrophy was the only lesion of thymus observed in our study. It is a universal finding in all aged rats (Burek and Meihuizen, 1977). In fact, the atrophy is so severe
that the thymus is very difficult to locate. Thymic sections were prepared by embedding the whole superior mediastinum after division into three pieces. With this procedure, we were able to examine the thymus histologically in one-third to one-half of the rats in our study. No other non-neoplastic lesions of thymus are known to occur in rats. Various strains of aged rats develop a low incidence of thymomas, but tumors of other types are very rare (Altman and Goodman, 1979). No primary thymic neoplasias were observed in our study.

b) Lymph Node

Although lymph node lesions have not been reported to be more frequent in the human diabetic population than in nondiabetics, a variety of strain specific (but unrelated to diabetes) lymphadenopathies were nearly ubiquitous in our BBW rats. This is in stark contrast to the nearly homogenous, normal appearance of the lymph nodes in our W rat population. Rats have multiple mesenteric lymph nodes at the junction of the cecum and the colon. In W rats these were small (2-4 mm diameter) and distinct, while in BBW rats they were usually fused into one or more longitudinal nodes, some of which were very large (Figure 4.30). Although there was no uniform histological appearance in these longitudinal nodes, most had some form of sinusoidal hyperplasia. Three distinct cell populations predominated in these nodes. In
some, sinusoids were massively dilated with plasma cells and differed from plasmacytomas only by the presence of occasional cortical follicles. Others were consistent with the diagnosis of sinus histiocytosis. Still other rats had nodes with mixed cell types in the dilated sinusoids. Regardless of the type of sinusoidal hyperplasia, germinal centers were rarely seen in the cortical follicles. Lymph nodes from the W rats in our study had distinct follicles, frequently with germinal centers (Figure 4.31), and never showed the sinusoidal hyperplasia typical of BBW rats (Figure 4.32).

Granulomatous lesions were not uncommon (3-5%) in BBW rats (Table 4.5), but were never seen in W rats. Grossly, these nodes were white, non-hemorrhagic, firm, and frequently fused together. In no case could organisms be demonstrated in these granulomas (Figure 4.33).

Although aged rats are less immunocompetent than younger rats, age-related morphological changes within the lymph nodes are usually minimal. Lymph nodes from aged rats are grossly normal (Pollard and Kajima, 1970); the general histological trend with age is toward a "less active" appearance with a decrease in the number of germinal centers per node. Frequently the medullary cords are densely packed with plasma cells that may extend into the paracortical region. Hemosiderin-containing macrophages are often present in the sinuses (Burek, 1978). This appears to be a
physiologic change associated with aging rather than a pathologic change. The only common lesions in the nodes of aged rats are cystic changes in the sinusoids (Anver and Cohen, 1979; Burek, 1978).

Lymphomas were the predominant neoplastic lesions observed in our study. They were found in a modest percentage (3-4%) of older BBW rats (Table 4.5), but were not seen in W rats. The mesenteric lymph nodes and the colon were the most frequent sites of involvement (Table 4.6). Grossly most were firm, lobulated, white, and severely hemorrhagic (Figure 4.34). Histologically, lymphomas were diffuse (Figure 4.35), and were "histiocytic" with plasmacytoid differentiation (Figure 4.36). Colon involvement occasionally caused severe bowel obstruction (Figures 4.37 and 4.38). In one rat (Figure 4.39) the gross appearance of the lymphomas was that of a focal ulceration of the intestinal mucosa without any associated nodal involvement. Kalant and Seemayer (1979) have previously reported a 12.6% and 1.2% incidence of mesenteric lymphomas in BBWd and BBWzd rats respectively. These tumors were histologically either plasmacytoid or had features of immunoblastic sarcomas. Cytoplasmic immunoglobulin was demonstrated by immunoperoxidase confirming their B-cell origin (Seemayer et al., 1982b; Seemayer et al., 1983). We are not aware of any studies on lymphoproliferative disease in BBW rats since 1983. Using the "Working Formulation for
Clinical Usage" classification scheme, these lesions are now best classified as large cell immunoblastic lymphomas. Interestingly, 50% of B-immunoblastic lymphomas in man are associated with a previous history of autoimmune disorders or systemic immunodeficiency (Cotran et al., 1994).

A wide variety of neoplastic lesions have been reported to occur sporadically in aged rats. These include lymphomas, lymphosarcomas, lymphoreticular tumors, reticulum cell sarcomas, reticulosarcomas, and plasmacytomas (Altman and Goodman, 1979). It is difficult to make generalizations about their incidence because the literature describing many of these predates modern systematic description of hematopoietic system tumors. Therefore, tumor classifications are ambiguous (Snell, 1963); some investigators group all primary lymph node tumors as lymphomas. Thompson et al. (1961) found one lymphoma in a series of 125 Sprague-Dawley rats. Ratcliffe (1940) observed only one lymphoma in a series of 468 Wistar rats. Olcot (1950) and Kim et al. (1960) also reported very low incidences of lymphomas. Most large studies have reported none (Davis et al., 1956; McCoy, 1909; Boorman and Hollander, 1973; Burek, 1978; Snell, 1963). Crain (1958) reported a higher incidence of "malignant lymphomas" in his study but his classification system is ambiguous. It seems likely that the rate of spontaneous lymphomas in aged outbred rats is less than 1%. 
Lymph node neoplasms are apparently most common in 2-3 year old rats (Burek and Hollander, 1977; Boorman and Hollander, 1973; Ueberberg and Lutzen, 1979) and have not to my knowledge been previously reported in rats less than 17 months of age (Burek and Hollander, 1977). Since the youngest rat mentioned in the literature with a true lymphoma is a 20 month old Sprague-Dawley rat (Thompson et al., 1961), it is interesting that BBW rats develop lymphomas at such a young age.

c) Spleen

The incidence of splenic lesions in human diabetics has not been reported in the literature, but there is little reason to expect any significant change from normal. Some older sources claimed that there is an increased lipid content in the spleens of diabetics. If this is true, it is a minimal increase (Warren et al., 1966).

Age-related pathological changes in the spleen of the rat are infrequent as well (Burek, 1978). One study (Andrew, 1946) did report some minor morphological changes in the spleens of old rats including less discrete Malpighian corpuscles, absence of germinal centers, and a more sinusoidal arrangement of the red pulp with an increase in hemosiderin-filled macrophages. Megakaryocytes may also be more common. This picture was occasionally seen in some of the older BBW rats (Figure 4.40), but was not observed in
our W rats.

Mild or moderate extramedullary hematopoiesis was the only common splenic finding and was present in greater than one quarter of the rats in this study regardless of strain. Although mild extramedullary hematopoiesis is a very common finding in many strains of rat, it is most frequently severe in tumor-bearing animals (Anver and Cohen, 1979). In our study, BBW rats with lymphomas frequently had severe splenomegaly secondary to extramedullary hematopoiesis and/or tumor infiltration (Figure 4.41). It is interesting that extramedullary hematopoiesis was very rare at any other site in our study.

Altman and Goodwin (1979), in their review of neoplasia in rats, were able to find only two reports of splenic tumors in rats. No primary tumors were found in this study.

10. Nervous System
   A. Brain

   Although untreated hyperglycemia has severe functional effects on the peripheral nervous system, the central nervous system is relatively unaffected. On the other hand, hypoglycemia severely affects the CNS but usually has very little effect on the peripheral nerves (Brierly, 1981). Hypoglycemic brain damage has been seen as a result of insulin shock therapy for psychoses, irreversible coma after treatment of diabetes with insulin, insulinomas, infants of
diabetic mothers and idiopathic hypoglycemia in infants. Hypoglycemic brain damage morphologically bears a similarity to hypoxic brain damage. Pyramidal neurons in Sommer's sector of the hippocampi become necrotic and diffuse demyelination of the centrum ovale occasionally is seen. When the cerebellum is involved, the damage is predominantly to the Purkinje cells (Brierly, 1981; McCall, 1992).

Although pure hyperglycemia appears to have little adverse effect on the brain, diabetic ketoacidosis can lead to massive diffuse cerebral edema as well as uncal and tonsillar herniation. The mechanism for this typically fatal complication is unknown. Suggested contributing factors include rapid correction of blood glucose and/or hyponatremia, increased CSF pressure due to increased polyol pathway activity in the brain, and cerebral hypoxia (McCall, 1992).

Finally, diabetes predisposes patients to thrombotic (but not hemorrhagic) cerebrovascular disease. The risk of stroke is two-to-six-fold increased. Diabetes is believed to be responsible for at least 7% of stroke deaths, and cerebrovascular disease is seen in approximately 25% of patients dying with diabetes. The mechanism of this increased risk is unknown but is in part related to the increased risk of atherosclerosis and hypertension in diabetes (McCall, 1992; Standl et al., 1989).
CNS lesions were very uncommon in our study, and when they were present were usually iatrogenic in origin. Hypoglycemic brain damage (Agardh et al., 1981) was observed in two BBWd rats as a result of excessive insulin therapy. In both rats, convulsions preceded their sacrifice so hypoxia may have been a factor in addition to hypoglycemia. Both rats showed eosinophilia of the pyramidal neurons in Sommer's sector of the hippocampus. The Purkinje cells of the cerebellum showed eosinophilia in one of the rats.

The most interesting CNS finding was a lesion very similar to central pontine myelinolysis (CPM), a lesion in man characterized by a single focus of demyelination in the pons with relative sparing of nerve cells and axons. CPM is most frequently seen in alcoholism and malnutrition and is now believed to be an iatrogenic disorder due to the rapid correction of hyponatremia (Wright et al., 1979). This is a relatively rare disorder in man and has not been previously induced in experimental animals. However, Kleinschmidt-DeMasters and Norenberg (1982) have shown that the rapid correction of hyponatremia can cause a relatively diffuse demyelination of the brain in the rat.

CPM was observed at necropsy in an emaciated 333 day old BBWd rat that had been treated for dehydration by subcutaneous injection of a total of 15 mls of normal saline over a period of one week (Wright et al., 1983c, appendix G). We can only speculate as to why CPM might occur in a
BBW diabetic rat yet has not been produced experimentally or observed incidentally in other animals. First, CPM is more common in chronic nutritionally deficient individuals. Poorly controlled diabetes mellitus is certainly such a condition. The emaciation and dehydration that frequently precedes the death of BBW diabetic rats may set the stage for the development of CPM. Secondly, the diabetic state causes additional osmotic stress. Aloia and Nilakantan (1973) have shown that fluctuations in the cerebral spinal fluid (CSF) sodium level are responsible for osmotic equilibrium between the CSF and the plasma. This prevents the abrupt fall in plasma glucose and osmolality following insulin treatment resulting in a shift of water into the brain (i.e. elevating CSF pressure). We postulate that the combination of these conditions followed by rapid treatment with relatively large doses of saline resulted in CPM in this case. Regardless of the mechanism, CPM has been reported in man several months after the onset of clinical diabetes and concurrent with fluid therapy for dehydration (Behar et al., 1964). A second probable case although less severe than the first, was seen in another BBWd rat, but treatment sheets were not available for that animal.

In addition to these iatrogenic lesions, a single case of acute meningitis was seen in a 289 day old BBWd rat. Even though the incidence of inflammatory lesions of the ears and nose is high in most rat colonies, reports of
meningitis or other CNS infections in rats are relatively rare (Bullock et al., 1968). In fact, reports of central nervous system lesions in most strains of rats are exceedingly rare (Anver and Cohen, 1979), particularly before 18 months of age (Coleman et al., 1977). In the present study, no CNS lesions were observed in BBWnd or W rats. Anver and Cohen (1979) claim that the only clinically significant CNS lesion in rats is degenerative myelopathy secondary to radiculoneuropathy. Although this is common in many strains of aged rats, the etiology is unknown. All other lesions appear to be incidental findings.

The most commonly reported age-associated brain lesion in rats is idiopathic vacuolation of the white matter of the thalamic area, pons, midbrain, and cerebellum (Burek, 1978; Anver and Cohen, 1979; Coleman et al., 1977). In fact, Coleman et al. (1977) reported an 80% incidence rate in Fischer 344 rats over 30 months of age.

High frequencies of various pigments have been reported (Burek, 1978). He observed a nearly 100% frequency of neuronal accumulation of intracytoplasmic lipofuscin in rats over 24 months of age. He also frequently found melanosis of the meninges and olfactory lobe of the brain. Melanosis was apparently not age associated as it was seen in both young and old rats. Burek (1978) also reported PAS-positive laminated extracellular basophilic bodies in thalamus or grey matter of the cerebellum of 20% of his rats.
addition to these, age related membranous bodies were reported in the dendrites of cerebral cortical neurons of Sprague-Dawley rats (Anver and Cohen, 1979). Infrequent or single observations of hydrocephalus, atherosclerosis, infarction, focal hemorrhage, focal hemorrhagic necrosis, ischemic necrosis, and multifocal spongiform encephalopathy have been reported (Burek, 1978 and Coleman et al., 1977).

A wide variety of brain tumors has been reported in various aged inbred or outbred rat strains, but the incidence of most of these is under 1%. Hollander et al. (1976) reported 12 primary granular cell myoblastomas in various strains of aged inbred rats. Ueberberg and Lutzen reported 9 glioblastomas in rats over 21 months of age and that male rats were more frequently afflicted. Coleman et al. (1977) were unable to find any CNS tumors in 144 male Fischer 344 rats of variable age. Burek (1978) reported a less than 1% incidence of ependymomas and oligodendrogliomas in BBW/Bi rats and of astrocytomas, glioblastoma multiforme, and meningiomas in WAG/Rij rats. All tumors were present in rats between 22 and 41 months of age. He also reported a 2-7% incidence of granular cell tumors in these rats. Dagle et al. (1979) examined a total of 2,242 aged Wistar, Sprague-Dawley, and Osborne-Mendel rats and reported 1.3%, 1.2% and 1.9% incidences respectively. They reported predominantly astrocytomas but also observed several malignant ependymomas, meningiomas, pinealomas, neoplastic
reticulosis, as well as an isolated oligodendroglioma and a gliomatosi. No CNS neoplasms were observed in our study.

B. Peripheral Nerve

Peripheral neuropathy is a common sequella of long-standing diabetes mellitus (Greene et al., 1989; Greene et al., 1992). This entity has been extensively studied in BB Wistar rats (Sima, 1980; Sima and Hay, 1981; Sima and Thibert, 1982; Sima et al., 1982; Sima, 1983; Sima et al., 1983; Sima and Hinton, 1983; Greene et al., 1984; Sima, 1985; Yagihashi and Sima, 1985a; Yagihashi and Sima, 1985b; Sima et al., 1986; Mendell et al., 1980; Mendell et al., 1981).

11. Other

Various other organ systems are also adversely affected by the diabetic state. In this study, gross observations on other organ systems in the BB Wistar rat will be briefly presented. These organ systems include the skin, eyes, and adipose.

a) Cutis

Approximately 30% of long term human diabetics develop cutaneous lesions as a result of microangiopathy, arteriosclerosis, infection, metabolic disturbances, and/or the sequelae of chronic therapy. Integumentary diseases
associated with diabetes mellitus include: benign acanthosis nigricans, Kyrle's disease (hyperkeratosis follicularis et parafollicularis in cutem penetrans), necrobiosis lipoidica diabeticorum, ruberosis, carotenemia, scleredema, granuloma annulare, skin spots, and bullous diabeticum. In addition to these, improper insulin treatment may result in cutaneous disorders. The most frequent of these is insulin lipodystrophy (Huntley, 1993; Perez and Kohn, 1994).

Skin disorders were infrequently observed in the BBW rat, but were not routinely examined with histology. A large proportion of the offspring of several litters became bald over the head, shoulders, and thighs. The skin appeared normal but with less than the normal amount of hair. The lesions were too bilaterally symmetrical for a parasitic infection and the pattern was suggestive of a temporary endocrine disorder (R. Bell, personal communication). All rats outgrew the disorder by approximately 90 days of age. Cutaneous ulcerations, seen very infrequently, were the other main integumentary lesions observed in BBW rats.

b) Eyes

Ocular disorders are some of the most frequent and disastrous complications of human diabetes mellitus. In fact, diabetic retinopathy is considered the leading cause
of blindness in adults under age 65 in the United States (Kohner, 1989). In addition to diabetic retinopathy, diabetics also have a higher incidence of cataracts and glaucoma. Occular histology of the BBW rat was not studied, but gross occular abnormalities observed in the BBW rats included cataracts, microphthalmia, and corneal ulceration. The total incidence was less than 5%. There is also some evidence that BBWd rats develop lesions resembling diabetic retinopathy (Sima et al., 1985), but this was not examined in our study.

c) Soft tissues

One commonly-mentioned advantage of the BBWd rat as a model for juvenile-onset diabetes is the absence of obesity (Nakhooda et al., 1978). In fact, the animal appears somewhat emaciated when compared side-by-side with a Wistar rat of the same general body proportions. The anatomical sites that store excess fat in the Wistar rat (e.g. - epididymal fat pat, omentum, etc.) grossly contain less adipose tissue in the BBW diabetic rat. This is undoubtedly due to the limited insulin regimen and severe diabetes (4+ glucose) in our BBWd rats and is consistent with the emaciated appearance often seen in untreated human IDDM. Other studies have suggested that adipose differs from most other tissues by not showing capillary basement membrane thickening in diabetics (Warren et al., 1966).
One soft tissue tumor, a subcutaneous spindle cell sarcoma, was identified in a BBW rat of unknown age (Figure 4.42).

B. Hematology

Until relatively recently, it was believed that there were no specific hematological changes in diabetes mellitus, because there are no characteristic morphological abnormalities in peripheral smears or bone marrow aspirates. However, metabolic studies have found a vast array of metabolic and biochemical abnormalities, but the clinical significance of many of these is unknown.

Erythrocyte studies have demonstrated a relationship between the concentration of glycosylated hemoglobin (A1c) and the duration of hyperglycemia (Cole, 1978; McDonald and Davis, 1979). Glycosylation also suggests a mechanism for the development of basement membrane thickening as a sequelae of diabetes mellitus (Jones and Peterson, 1981). Other findings pertaining to the diabetic RBC include abnormal oxygen affinity, glycosylation of the 2,3-diphosphoglycerate binding site, decreased concentration of inorganic phosphate, increased viscosity, and decreased deformability (Jones and Peterson, 1981).

The numerous white blood cell (WBC) defects that have been reported to be associated with diabetes mellitus have been reviewed by Jones and Peterson (1981).
Polymorphonuclear neutrophil (PMN) defects include abnormal adherence, migration, chemotaxis, phagocytosis, and killing. Several enzymatic abnormalities have also been reported but alteration in the number of PMNs is not a characteristic of diabetes. Lymphocytes, particularly T-cells, have been shown to have abnormal metabolic properties, mitogen cell responses, and cell surface properties in both human and animal diabetes. The presence of defects in the eosinophils or basophils of diabetics has not been adequately studied to justify any conclusions. It is likely that some of these defects are responsible for the increased susceptibility of poorly-controlled diabetics to infection (Robertson and Polk, 1974).

There is good reason to suspect platelet abnormalities in diabetes. Some evidence suggests that the hemostatic system may be involved in the initiation or propagation of atherosclerotic lesions in diabetics (Tschoepe et al., 1993). In fact, frequently the results of in vitro studies seem to have little in vivo significance. Although it is well established that there is no significant difference in platelet counts or platelet survival between diabetic patients and control subjects, hyperglycemia does lead to a hypercoagulable state, but this cannot be observed with standard clotting assays (e.g. prothrombin time, activated partial thromboplastin time, and thrombin time) (Glassman, 1993).
In summary, there do appear to be hematological abnormalities associated with diabetes, but it is presently uncertain whether many of these have any clinical significance.

In the present study, there were many more strain-related differences (those present in both BBWd and BBWnd but not in control) than diabetes-related (those in BBWd but neither BBWnd nor control) differences (Wright et al., 1983d, appendix H). More specifically, the BBW strain had significantly decreased numbers of white cells and platelets, as well as markedly changed differential white cell counts. Differential counts revealed a pattern of marked lymphopenia, slight neutrophilia, monocytosis, and eosinophilia. Jackson et al., (1981) have shown that this marked lymphopenia is due to a T-cell deficiency and Greiner et al. (1986) have shown that this is due to depletion of the RT-6+ T-cell subset. No differences in gamma globulin concentrations were observed. Some statistically significant changes in red cell indices were seen, but none were outside of the normal range of other rat strains (Ringler and Dabich, 1979). Red cell distribution width (RDW) values were significantly higher in the BBW strain (particularly BBWd), indicating a tendency toward anisocytosis. These strain-related changes are consistent with present knowledge since inbreeding of rats is known to change hematological parameters (Hulse, 1965) and since
diabetes does not significantly alter major red and white cell indices in man (Jones and Paterson, 1981).

The changes in red cell indices indicate either a sex-related or random pattern and none of these values are very far out of ranges considered normal for rat hematological studies (Ringler and Dabich, 1979).

Most of the white blood cell indices are changed in a strain-related pattern. Both BBWd and BBWnd have significantly lower white cell counts than control rats as a result of lymphocytopenia. This could partially explain the higher incidence of infections (Wright et al., 1980; Sima, 1980) and possibly the apparent shortened life expectancy of the BBW rat (unpublished observation). Reich and Dunning (1941) have reported a positive correlation between the mean life-span of inbred rat strains and their mean WBC count.

Marked eosinophilia was present in many of the BBW rats regardless of the presence of diabetes. Since no parasitic infections could be demonstrated, hypersensitivity to some unknown allergen or autoantigen may be responsible. This is supported by the observation that massive numbers of eosinophilic infiltrates in the tissue sections of some rats in our study (Figure 4.43). Eosinophilic infiltrates in the islets of some rats have also been reported (Nakhooda et al., 1977). This is interesting in light of the frequent occurrence of peri-insular and insular eosinophilic infiltrates in the offspring of diabetic mothers (Barresi et
al., 1978).

The other major strain-specific observation in this study was that BBW rats have decreased platelet concentrations relative to W rats (p <0.01) (Table H.1). It is questionable whether a change of this small magnitude would have any effect on the process of atherogenesis in BBW rats.

The mean gamma globulin concentrations in our study are well within the normal ranges reported in other studies (Ringler and Dabich, 1979; Coleman et al., 1977) and no group differences in the concentrations of gamma globulins were observed (Wright et al., 1983d). Therefore, hypogammaglobulinemia cannot account for the increased susceptibility to infection in the BBW strain. However, it is not possible to rule out some abnormality in the distribution of immunoglobulin fractions as an etiological factor.

C. DNA Repair and Longevity

The BBW rat appears to have a significantly shortened lifespan relative to other inbred or outbred rats. The oldest BBW rats in our study were a 496 day old BBWnd female and a 465 day old BBWd male which was non-diabetic until 38 days before its death. The average lifespan of the outbred Wistar rat maintained in standard animal housing is about 26 months (Hoffman, 1979). The maximum achievable lifespan for
an outbred specific pathogen free Wistar rat is about 46 months (Hoffman, 1979). The mean age (± standard deviations) of BBWd (n=95) and BBWnd (n=12) rats that died spontaneously in this study were 274.5 ± 87.1 days and 288.6 ± 142.6 days, respectively. No Wistar rats died spontaneously in this study.

Since it seemed likely that the high incidence of pneumonia in BBW rats might have lowered these age values rather significantly, I calculated these lifespans again excluding rats with pneumonia and found that the rats with pneumonia lived on average much longer than those without it. This is probably because pneumonia occurs selectively in older rats. Therefore, it is necessary to postulate other causes for this apparently shortened life expectancy in BBW rats. Undoubtedly, some of these animals died because of inappropriate insulin treatment, but this does not explain the similar trend in BBWnd rats.

A key question was to determine whether or not there is a genetic basis for the decreased longevity in the BBW strain. Since it is well known that maximal achievable lifespan correlates directly with cellular DNA repair capacity (Hart and Setlow, 1974), we compared the cellular DNA repair capabilities of the BBW strain and that of the outbred Wistar strain from which they were originally derived. (Wright et al., 1986, appendix J). In this study we compared ultraviolet (UV) induced excision (long-patch)
repair in the two strains because this type of repair requires endonuclease, exonuclease, polymerase and ligase activities. Therefore, it should provide a sensitive screen for problems with DNA repair.

As shown in Figure I.2, unscheduled DNA synthesis, a measure of excision repair, did not significantly differ between the two strains. Therefore, this cannot explain the higher incidence of degenerative, autoimmune, and neoplastic diseases nor the decreased longevity in this strain.
## TABLE 4.1

### INCIDENCE OF HEPATIC LESIONS BY AGE

<table>
<thead>
<tr>
<th>LEOSON</th>
<th>BBWd RATS</th>
<th>BBWnd RATS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age in days</td>
<td>age in days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-120 n=8</td>
<td>121-240 n=42</td>
<td>241-360 n=59</td>
<td>361-480 n=18</td>
</tr>
<tr>
<td>Normal</td>
<td>8 (68.9%)</td>
<td>35 (68.9%)</td>
<td>51 (66.4%)</td>
<td>13 (72.2%)</td>
</tr>
<tr>
<td>Fatty Change</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Accessory Lobe</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Marked bile duct hyperplasia</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Infarction</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (22%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Congestive necrosis</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Abscess</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

A total of 45 W rats were examined. Isolated findings included fatty change (2.2%), infarction (2.2%), and congestive necrosis (2.2%).
### TABLE 4.2

**INCIDENCE OF PULMONARY LESIONS BY AGE**

<table>
<thead>
<tr>
<th>LESION</th>
<th>BBWd RATS age in days</th>
<th>BBWsd RATS age in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-120</td>
<td>121-240</td>
</tr>
<tr>
<td>Normal</td>
<td>8 (100%)</td>
<td>34 (69.4%)</td>
</tr>
<tr>
<td>Broncho pneumonia</td>
<td>0 (0%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>Hemorrhagic pneumonia</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Aspiration pneumonia</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Lobar pneumonia</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Granuloma</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Except for two instances of bronchitis, no pulmonary lesions were observed in BBWsd rats.*
### TABLE 4.3

**INFECTIONOUS ORGANISMS ISOLATED FROM BBW RAT TISSUES**

<table>
<thead>
<tr>
<th>PULMONARY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacilli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td></td>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Cocci</td>
<td>Staphylococcus (coag. neg.)</td>
</tr>
<tr>
<td></td>
<td>Streptococcus, alpha</td>
</tr>
<tr>
<td></td>
<td>(histochemically not group D)</td>
</tr>
<tr>
<td></td>
<td>Streptococcus faecalis</td>
</tr>
<tr>
<td></td>
<td>Veillonella parvula</td>
</tr>
<tr>
<td>OTHER SITES</td>
<td></td>
</tr>
<tr>
<td>Bacilli</td>
<td>Clostridium perfringens</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
</tr>
<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td></td>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td>Fungus</td>
<td>Candida sp. not albicans</td>
</tr>
<tr>
<td>LESION</td>
<td>BBWd RATS age in days</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td>0-120 n = 7</td>
</tr>
<tr>
<td></td>
<td>121-240 n = 39</td>
</tr>
<tr>
<td></td>
<td>241-360 n = 52</td>
</tr>
<tr>
<td></td>
<td>361-480 n = 18</td>
</tr>
<tr>
<td></td>
<td>unknown n = 1</td>
</tr>
<tr>
<td></td>
<td>total n = 117</td>
</tr>
<tr>
<td></td>
<td>total n = 22</td>
</tr>
<tr>
<td>Normal</td>
<td>7 (100%)</td>
</tr>
<tr>
<td></td>
<td>(97.4%)</td>
</tr>
<tr>
<td></td>
<td>(98.1%)</td>
</tr>
<tr>
<td></td>
<td>(72.2%)</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
</tr>
<tr>
<td>Myocardial degeneration</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0 (1.9%)</td>
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<tr>
<td></td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>3 (100%)</td>
</tr>
<tr>
<td></td>
<td>4 (3.4%)</td>
</tr>
<tr>
<td>Acute myocarditis</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0 (2.6%)</td>
</tr>
<tr>
<td></td>
<td>0 (11.1%)</td>
</tr>
<tr>
<td></td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Endocardial proliferation</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0 (2.6%)</td>
</tr>
<tr>
<td></td>
<td>0 (1.7%)</td>
</tr>
<tr>
<td></td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*Myocardial degeneration was present in 2 of 5 (40%) W rats in the 481-600 day old age group but was not present in any younger W rats. No other cardiac lesions were observed in W rats.
TABLE 4.5

INCIDENCE OF LYMPHATIC LESIONS BY AGE*

<table>
<thead>
<tr>
<th>LESION</th>
<th>BBWd RATS</th>
<th>BBWnd RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age in days</td>
<td>age in days</td>
</tr>
<tr>
<td></td>
<td>0-120 n = 8</td>
<td>121-240 n = 51</td>
</tr>
<tr>
<td>Normal</td>
<td>8 (100%)</td>
<td>47 (92.2%)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
</tr>
<tr>
<td>Granuloma</td>
<td>0 (0%)</td>
<td>3 (6.0%)</td>
</tr>
</tbody>
</table>

*No lymphatic lesions were observed in W rats.
TABLE 4.6

SITES OF LYMPHOMATOUS INVOLVEMENT

<table>
<thead>
<tr>
<th>GROUP</th>
<th>RAT #</th>
<th>SEX</th>
<th>AGE</th>
<th>MES.</th>
<th>MED.</th>
<th>OTHER</th>
<th>THYMUS</th>
<th>COLON</th>
<th>S.I.</th>
<th>LIVER</th>
<th>PANC.</th>
<th>SPLEEN</th>
<th>LUNGS</th>
<th>OVARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBWd</td>
<td>015</td>
<td>M</td>
<td>248</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>235</td>
<td>M</td>
<td>332</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>241</td>
<td>F</td>
<td>198</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>+</td>
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<td></td>
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<td>+</td>
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<td>+</td>
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<tr>
<td></td>
<td>1213</td>
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<td></td>
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<td>+</td>
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</tr>
<tr>
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</table>

abbreviations: mes., mesentery; med., mediastinum; s.i., small intestine; panc., pancreas
CHAPTER V

GENERAL DISCUSSION (SUMMARY)

Throughout this entire study, incidences of various lesions and other findings have been tabulated by group (BBWd, BBWnd, and W) and by age. In order to understand the significance of these lesions, these results have been classified as follows (Wright et al., 1983e, appendix J):

I. Strain-related lesions are those abnormalities found in BBWd and BBWnd rats but not in W rats.

II. Diabetes-related lesions are those abnormalities found in BBWd rats but not in BBWnd or W rats. The lesions are subdivided into three groups:
   a. lesions strongly associated with human diabetes.
   b. lesions weakly associated with human diabetes.
   c. lesions not specifically associated with human diabetes.

III. Rat-related lesions are those that occurred in BBWd, BBWnd, and W rat with similar frequencies.

IV. Organ systems virtually free of significant pathology.
Table J.1 shows the incidence of strain-related lesions found in BBW rats. These include lymphoproliferative, inflammatory and developmental disorders. The presence of lymphoid hyperplasia, spontaneous granulomas in the absence of demonstrable organisms, lymphomas, eosinophilia, and lymphocytopenia all suggest an abnormal immune system. The high incidence of infections in BBW rats is consistent with this.

Tables J.2A and J.2B show the incidence of diabetes-related lesions that are strongly associated with diabetes (insulitis, testicular atrophy, and cataracts) and those that are weakly associated with diabetes (hepatic fatty change, pancreatitis, lymphocytic thyroiditis, hypoglycemic brain damage, central pontine myelinolysis). The presence of these human diabetic sequelae in BBWd rats supports the validity of the model.

Table J.2C also includes several lesions that are diabetes-related in the rat, but not specifically associated with diabetes in man. This category is comprised of stomach erosions and idiopathic megacolon. Lesions in this category, as well as those in the strain-related category, are undesirable and uncontrollable variables that may affect experiments utilizing this model. Of these, most are minor inconveniences because of either low incidences or minimal deleterious effects. Only the pulmonary infections and
gastric erosions are likely to be of practical consequence for most experiments. Low incidences of several other types of lesions (i.e. - myocarditis and hepatic infarction) appeared in all groups of rats.

Finally, the absence of several important sequelae of human diabetes (i.e. - diabetic nephropathy, atherosclerosis, and severe microangiopathy) suggests a degree of infidelity as a model for human diabetes mellitus.

Comparison of the findings in this study with the incidence of lesions in other strains of "aged" rats tends to downplay the significance of some of the lesions in the BBW rats. Generally speaking, only rats in excess of two or two and a half years of age are considered "aged". By this standard, none of the BBW rats in this study are "aged" because they do not live that long. Most outbred rat strains do not develop any significant lesions (except chronic respiratory infections) until they are over two years of age. This explains the paucity of lesions in our W rat series. The occurrence of a high incidence of typical senile lesions in the relatively young BBW rats suggests some mechanism for premature aging could be involved.

An important point that may be easily overlooked is that all of the lesion incidences are characteristic of BBW rats housed under the conditions present in the semi-barrier facilities at Wiseman Hall at The Ohio State University. It
is possible that animals housed elsewhere under different conditions may have somewhat different characteristics. This is particularly true since many of the BBW colonies around the country were started from a few mating pairs of outbred rats shipped from Ottawa and then were subsequently inbred by sibling mating within the new institutions. This geographic isolation would tend to firmly fix and amplify unusual traits (i.e. - similar to the process of speciation). In addition to inbreeding, the environmental conditions at the new institutions can have a significant effect on the expression of traits, even in highly inbred strains of laboratory animals. For example, 100% of inbred C3H-A'y mice developed both mammary and liver tumors when housed in the United States, but when this same colony was transferred to Adelaide, Australia, only 17% of the mice developed liver tumors. When food and bedding were imported from the United States to Australia, the 100% incidence was re-established (Sabine et al., 1973).

How Good is the BBW Model? What is a Model?

A model is something that represents something else. Several features are common to most models: (1) No model is perfect. If it were, then it would be the original. Therefore, studies performed on models are less meaningful than studying the original. (2) There is no way to know
how good a model really is. Even if all examinable features of a model and the original appear to be the same; there is no guarantee that similar mechanisms are involved in producing those similarities. (3) There is an advantage to using multiple models. If results from several different models agree, this suggests that a similar mechanism may be involved. (4) Since results based on the use of models are less meaningful, models are used predominantly when the "cost" of studying the original is too great (i.e. - financial, technical, ethical, or legal reasons prevent studying the original but are not so limiting to studying the model). (5) Although models are only useful in so much as they mimic the real thing, differences can sometimes be exploited when trying to understand mechanisms. For example, the question can be asked: "Why does the model behave differently than the original when they appear to be so similar?" Even with all of these inherent limitations, animal models still have made major contributions to the understanding and treatment of human diseases (Jones, 1980).

Animal models for human diseases are studied mainly because their environment, their heredity and their age or time of death can be more easily controlled than when dealing with humans. Table 5.1 shows the characteristics of a good animal model as outlined by Leader and Padgett (1980).
Based on these criteria, how good is the BBW rat as a model for IDDM? Several of the criteria are very easily satisfied by the BBW rat. First, it appears to reproduce accurately the metabolic features of the disease, the morphology of the disease, and some of the sequelae of the disease. Second, modest numbers of outbred rats are readily available to qualified investigators at no cost from Health and Welfare of Canada or larger numbers of inbred rats can be purchased from the University of Massachusetts colony. Third, it gives birth to multiple offspring—usually 5-10 pups per litter. Fourth, the BBW rat is docile and easily handled. Fifth, its size is greater than that of other rodent models. On the other hand, the BBW rat does not develop some of the major sequelae of diabetes, and it develops numerous other lesions or conditions that are not present in human diabetes that may serve as uncontrollable variables and may shorten the lives of the animals. Lymphocytopenia is an important example of the latter. Second, it is not very easily maintained. Daily insulin dosages must be carefully determined. A full-time skilled technician is required. Breeding problems are common, in part as a result of developing testicular atrophy at a young age. Third, the BBW rat appears to have a significantly shortened lifespan. The latter of these seems to be one of the most severe limitations of the model.
In conclusion, it is obvious that there are both advantages and disadvantages to the BBW model. As a result of our study, it is one of the best pathologically characterized models for diabetes research. It is hoped that this information will assist individual investigators to make an informed decision as to the suitability of the BBW rat for their experiments. This has become exceedingly important because of mounting pressure from both the scientific community and society to justify research using animal models. Societal and scientific norms agree that poorly designed research utilizing animals is unethical. However, animal models, if used appropriately, can provide important insights into many aspects of IDDM (Sieber and Traystman, 1993).
### TABLE 5.1

**CRITERIA FOR A GOOD ANIMAL MODEL (LEADER AND PADGETT, 1980)**

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<table>
<thead>
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<tbody>
<tr>
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<td>Accurately Reproduce the Disease and Sequelae</td>
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<td>Easily Maintained</td>
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<td>7.</td>
<td>Long Survival</td>
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FIGURE 4.1
Pituitary chromophobe adenoma in a 327 day old BBWd rat. The lesion was roughly spherical with a large central hematoma. Each large unit on rule is 1 cm.
FIGURE 4.2
Photomicrograph of the same adenoma. The tumor appeared somewhat pleomorphic with few mitotic figures. A portion of the neurohypophysis is seen on the left slightly above the lesion. (Reticulin, original magnification x25).
FIGURE 4.3
Inflamed seminiferous tubules in the periphery of an otherwise atrophic testis from a 397 day old BBWd rat. Tunica albuginea is markedly thickened. Rat had bilateral orchitis (HE, original magnification x25).
FIGURE 4.4

BBWd rat with multifocal granulomatous lesions. Several necrotic foci are present. All seminiferous tubules were totally devoid of germ cells and Sertoli cells. Thickening of tunica albuginea is also present (HE, original magnification x25).
FIGURE 4.5
Granuloma with several multinucleated giant cells (HE, original magnification x250).
FIGURE 4.6
Acute prostatitis in a BBWd rat (HE, original magnification x25).
FIGURE 4.7
Normal prostate from 551 day old W rat (HE, original magnification x25).
FIGURE 4.8
Normal prostate from 551 day old W rat (HE, original magnification x100).
FIGURE 4.9
Normal prostate from 551 day old W rat. Epithelium is columnar and negative image of the golgi is visible at the apical end of the cell (HE, original magnification x630). Lumens contain pale eosinophilic secretions.
FIGURE 4.10
Inactive prostate from 177 day old BBWd rat. Epithelium is low cuboidal to simple squamous. No secretion in the lumens. (HE, original magnifications x25).
FIGURE 4.11
Inactive prostate from 177 day old BBWd rat. Epithelium is low cuboidal to simple squamous. No secretion in the lumens. (HE, original magnifications x100).
FIGURE 4.12
Large uterine stromal polyps in 372 day old BBWd rat. Histology showed squamous metaplasia and acute inflammation in the lumen. No inflammatory infiltrates were seen in the stroma. Each unit on rule is 1 mm.
FIGURE 4.13
Mammary adenocarcinoma found in milk line near base of tail of a 248 day old BBWnd rat. Tumor measures approximately 3 cm. in diameter. Tumor was firm, white, and lobulated. No metastases were seen. Photograph was taken after the tumor had been shaved to remove the hair.
FIGURE 4.14
Photomicrograph of the mammary adenocarcinoma. Epithelium has squamous elements. Ductules contain a deeply eosinophilic secretion (HE, original magnification x100).
FIGURE 4.15

Idiopathic megacolon in a 327 day old BBWd rat. The rat presented with abdominal distention and anorexia and was treated with enemas. Treatments were temporarily effective. At necropsy, the colon and caecum were observed to be distended (up to 5 cm in diameter) with hard fecal material. Nothing was found constricting the rectum when the pubic symphysis was removed. All tissue in the area of the rectum was examined histologically and was normal. The duration of diabetes in this rat was 205 days.
FIGURE 4.16

412 day old BBWd rat with erosions and ulcerations in both the thick-walled glandular portion and the thin-walled squamous portion of the stomach. The glandular portion is severely hemorrhagic. The lesions in the squamous forestomach are surrounded by white, raised borders.
FIGURE 4.17

Small erosion in the thin-walled squamous portion of the stomach (forestomach). Area of coagulation necrosis extends to the muscularis mucosa. Epithelial surface has sloughed. (HE, original magnification x100).
FIGURE 4.18
Severe hepatic fatty change in the region of several portal triads in a 150 day old BBWd rat. Duration of diabetes was only 57 days. (HE, original magnification x250).
FIGURE 4.19
Several cysts consisting of dilated tubules filled with eosinophilic proteinacious fluid were seen in the kidney of a 412 day old BBWnd rat. The cyst just to the right of center is associated with focal chronic inflammation of the tubule. This pattern was seen in several rats and is typical of some of the changes seen in chronic renal disease in the rat. Most cysts in rat kidneys were not inflamed. (HE, original magnification x100).
FIGURE 4.20

A small focal subcapsular granuloma in the renal cortex of a 200 day old BBWd rat (HE, original magnification x100).
FIGURE 4.21
Chronic interstitial nephritis in a 210 day old BBWd rat. Mononuclear infiltrates, interstitial fibrosis, tubular atrophy, and relative sparing of glomeruli are prominent. (HE, original magnification x100).
FIGURE 4.22
Gross specimens from different lobes of a 325 day old BBWd rat with severe bilateral bronchopneumonia. The cut surfaces of the specimens on the left and right reveal large abscesses. The two center specimens show pleural adhesions.
FIGURE 4.23
Severely congested, edematous lung from a 192 day old BBWd rat. Presence of neutrophils in the alveoli indicate bronchopneumonia (HE, original magnification x250).
FIGURE 4.24
Murine chronic respiratory disease in a young W rat. Main features seen in this photomicrograph are peribronchial lymphoid hyperplasia, perivascular lymphoid aggregates, and chronic pneumonitis (HE, original magnification x25).
FIGURE 4.25

551 day old W rat with limited multifocal chronic myocarditis-predominately in the left ventricle. Antischkow cells are prominent in the chronic infiltrate. This picture is typical of myocardial degeneration in aged rats. (HE, original magnification x250).
FIGURE 4.26

Pregnant female BBWnd rat (age unknown) with vasculitis in all organ systems. A necrotizing vasculitis was seen in the pancreas (HE, original magnification x250).
FIGURE 4.27
Several less involved vessels were seen in the submucosa of the stomach (HE, original magnification x100). Other lesions in this rat included a small organizing mural thrombus in the heart, disseminated intravascular coagulation in the kidney (see Figure 4.29), severe extramedullary hematopoiesis in the spleen, and an infarct in a mesenteric node. Russell bodies were very prominent in the lymph nodes. The rat was also pregnant.
FIGURE 4.28
Focal vasculitis in the colon of a 320 day old BBWd rat. Histiocytes are seen predominantly in the intima and media and eosinophils are in the adventitia. This rat also had systemic eosinophilia. (HE, original magnification x100).
FIGURE 4.29

Two glomeruli with large microthrombi in the kidney of a near-term pregnant BBWnd rat (unknown age) which died with disseminated intravascular coagulation (DIC) secondary to the widespread vasculitis. (HE, original magnification x400)
FIGURE 4.30

Hyperplastic mesenteric lymph nodes from 320 day old BBWd rat. Nodes are enlarged and fused. Normal nodes are small and discrete.
FIGURE 4.31
Typical lymph node from a 300 day old W rat with prominent follicular germinal centers and abundant lymphocytes (HE, original magnification x10).
FIGURE 4.32

Hyperplastic lymph node from a 412 day old BBWd rat. Sinusoidal dilatation and lymphocytic depletion are prominent histologic features. (HE, original magnification x10).
FIGURE 4.33
Necrotizing granuloma in a pancreatic lymph node from a 125 day old BBWd rat. Additional sections were stained with acid fast blue, GMS, and gram stain. No organisms were seen. (HE, original magnification x25).
FIGURE 4.34

Lymphoma mass in a 334 day old female BBWd rat found at the junction of the cecum and colon. Tumor is firm, lobulated, and hemorrhagic. Tumor was also present in colon and pancreas.
FIGURE 4.35
Histologically, the lymphoma is diffuse with a total loss of normal nodal architecture. (HE, original magnification x25).
FIGURE 4.36
The tumor is histiocytic with plasmacytoid features. Several binucleated plasma cells are shown as well as a mitotic figure in the lower left corner. (HE, original magnification x400).
FIGURE 4.37
This lymphoma was found in a 248 day old BBWd rat. The tumor mass was found at the junction of the ileum and cecum. The three specimens in the photograph are organized proximal to distal from left to right. The lumen is totally occluded in the specimen on the left. Each large unit on the rule is 1 cm.
FIGURE 4.38
Photomicrograph of the area on the left. The neoplasm in the submucosa of the ileum is a histiocytic lymphoma with plasmacytoid features. (HE, original magnification x25).
FIGURE 4.39
Single, small, well-circumscribed area of necrosis in the proximal ileum (50 cm from cecum) of a 315 day old BBWd rat. Normal mucosa seen to right of necrotic area. Histological examination revealed much necrosis and a histiocytic lymphoma with plasmacytoid features. The adventitia of the colon was also involved, but this was not grossly visible. Lymph nodes were grossly large and histologically hyperplastic, but no tumor was seen. Spleen showed severe extramedullary hematopoiesis, but was not markedly enlarged.
FIGURE 4.40
Lymphoid depletion in the spleen of a 320 day old BBWd rat. Distinction between red and white pulp is obscured. (HE, original magnification x25)
FIGURE 4.41

Spleen from a BBWnd rat with a lymphoma (age unknown).
Spleen measures 7 cm long and weighs 7.5 gms. Normal values are 2-3 cm and 0.5-0.75 gm. Splenomegaly is a result of severe extramedullary hematopoiesis and tumor infiltration.
FIGURE 4.42
Massive subcutaneous spindle cell sarcoma in a BBW rat of unknown age.
FIGURE 4.43
Massive eosinophilic infiltrate in the submucosa of the colon of a 350 day old BBWd rat. The muscularis externa is also involved. Eosinophilic infiltrates were also seen in the other organs of this rat. (HE, original magnification x100).
Appendix A
HISTOPATHOLOGICAL LESIONS IN THE PANCREAS OF THE BB WISTAR RAT AS A FUNCTION OF AGE AND DURATION OF DIABETES

By

J. WRIGHT, A. YATES, * H. SHARMA and P. THIBERT

The Ohio State University College of Medicine, Department of Pathology, Columbus, Ohio 43210, U.S.A. and Animal Resources Division, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2

INTRODUCTION

The BB Wistar (BBW) rat, a useful animal model for juvenile-onset diabetes (JOD), was developed in the late 1970s from an outbred line of Wistar rats at the Bio Breeding Laboratories in Ottawa, Canada by Nakhooda, Like, Chappel, Murray and Marliss (1977). The BBW diabetic syndrome occurs spontaneously in the absence of obesity and is characterized by hyperglycaemia, glycosuria, ketoacidosis, insulinopenia, glucagonaemia, and hyperlipaemia (Nakhooda et al., 1977; Nakhooda, Like, Chappel, Wei and Marliss, 1978). Although the morphology of the pancreatic islets has been extensively studied in 60- to 120-day-old BBW rats at or near the time of onset of diabetes (Nakhooda et al., 1977, 1978; Seemayer, Tannebaum, Goldman and Colle, 1982), there has been no published report describing the incidence of pancreatic lesions in older BBW rats. In this study we have examined the incidence of both endocrine and exocrine pancreatic lesions in BBW Wistar diabetic (BBWd) rats, their non-diabetic siblings (BBWnd), and outbred Wistar rats.

MATERIALS AND METHODS

A breeding colony of BBW rats was established in the Pathology Department at The Ohio State University from the parent colony at the Health Protection Branch of Health and Welfare of Canada. Detailed descriptions of our animal housing facilities and animal husbandry techniques have been reported previously (Wright, Yates, Sharma and Thibert, 1981). A necropsy study was initiated to determine the incidence and types of lesions in our colony. Some of the results of these necropsies have been described elsewhere (Wright, Sharma, Thibert and Yates, 1980; Wright, Yates, Sharma and Thibert, 1983a).

Complete necropsies were performed on 121 BBWd, 43 BBWnd, and 33 outbred Wistar rats. All major organs including pancreas were grossly examined and fixed in either neutral phosphate buffered formalin or Bouin's fluid. The pancreas was removed intact and spread over a piece of cardboard to maximize surface area during fixation. After fixation, specimens were removed from the cardboard, and divided into 2 portions. The entire pancreas and representative portions of other organs were

* Correspondence to: Dr A. J. Yates, Division of Neuropathology, The Ohio State University, 111 Upham Hall, 473 West 12th Avenue, Columbus, Ohio 43210, U.S.A.
processed for light microscopy. Tissue sections were routinely stained with haematoxylin and eosin (H and E). Paraffin sections of specimens suspected of antemortem infection were stained with acid fast and Gram's stain, and Gomori's methenamine silver (GMS). Multiple sections of all pancreas specimens were examined by light microscopy. Statistical analyses were by analysis of variance, z-test, or chi-square test.

RESULTS

Table 1 shows the incidence of inflammatory pancreatic lesions in BBWd, BBWnd, and W rats. Insulitis was the most common inflammatory pancreatic lesion in both BBWd (14·0 per cent) and BBWnd (16·3 per cent) rats and is characterized as a mononuclear cell infiltrate involving the islets. In the majority, the infiltrate was predominantly peri-insular (Fig. 1) but the most severe lesions also included marked invasion of the islets (Fig. 2). A few eosinophils were often present at the periphery of the inflamed islets.

The ages of BBWd rats with insulitis ranged from 107 to 317 days of age (mean ± S.D. = 164±61·9 days) but the change occurred most frequently in the younger age groups (Table 1). In the BBWd it was seen only in those rats that died relatively shortly after the onset of hyperglycaemia and so its incidence was inversely related to the duration of diabetes ($\chi^2 = 33·4$, d.f. = 5, $P<0·001$) (Table 1). The longest period of time following the onset of diabetes for which a BBWd rat still had severe insulitis was 69 days; the mean duration of diabetes for BBWd rats with insulitis when they died was 49·9 ± 16·9 days.

In the non-diabetic rats, there was no specific age predilection for insulitis. It was observed in BBWnd rats ranging from 96 to 422 days of age (mean ± S.D. = 306·5 ± 132·4). Only one BBWnd rat with insulitis was less than 120 days old.

Several other types of inflammatory lesions also occurred (Table 1). Chronic interstitial inflammation (inflammation with little acinar or isular involvement) was the most frequent of these, occurring in all 3 groups of rats at incidences between 11 and 14 per cent. No other types of pancreatic lesions were seen in outbred Wistar rats. In BBW rats chronic pancreatitis (chronic inflammation with diffuse, extensive acinar involvement), acute pancreatitis, acute and chronic pancreatitis, acute interstitial inflammation, and acute and chronic interstitial inflammation all occurred but were not common. In the acute lesions eosinophils were often present either as the predominant cell type (Fig. 3) or mixed with neutrophils. No age pattern was discernible for any of the inflammatory lesions.

Granulomas were occasionally observed in BBW pancreatic tissue (Table 1). In no instance could any organisms be demonstrated with Gram, GMS, or acid fast stains. In 2 rats, granulomas and insulitis were present in close approximation (Fig. 4). Infiltrates of eosinophils were frequently associated with granulomas. In one rat several granulomas consisting almost entirely of reticuloendothelial cells and eosinophils were present in the pancreas.

Islet morphology in BBWd rats without insulitis was markedly altered from that of young pre-diabetic BBW rats. Shortly after the onset of glycosuria, islets from BBWd rats typically contained numerous endocrine cells with acidophilic cytoplasm and pyknotic nuclei (Fig. 5). Occasionally, hyperplastic
### TABLE A.1

**AGE INCIDENCE OF PANCREATIC LESIONS IN BB WISTAR RATS**

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<thead>
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<th>Lesion</th>
<th>BBW'd rats 0-120 days</th>
<th>BBW'd rats 121-240 days</th>
<th>BBW'd rats 241-360 days</th>
<th>BBW'd rats 361-480 days</th>
<th>BBW'd rats 481-600 days</th>
<th>BBW'd rats Not known†</th>
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<td>52</td>
<td>18</td>
<td>1</td>
<td>122</td>
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</tr>
</tbody>
</table>

* Except for 4 rats with chronic non-specific interstitial inflammation, no pancreatic lesions were seen in 32 W rats.

† Precise age not known but all were adults, at least 120 days of age.

§ The incidence of insulitis in BBW'd rats was inversely related to the duration of diabetes ($\chi^2 = 53.41, d.f. = 5, P < 0.0001$). The incidence in the following duration groups was: 0 to 60 days (14 in 32; 61 to 120 days (3 in 26; 121 to 180 days (0 in 17); 181 to 240 days (0 in 25); 241 to 300 days (0 in 12); and 301 to 360 (0 in 8).

§§ Both findings present in same rat.
Fig. A.1 Insulitis in a 115-day-old BBW rat. The mononuclear infiltrate is predominantly peri-insular with minimal islet invasion. Surrounding acinar tissue is not involved. HE x 50.

Fig. A.2 Islet from a 135-day-old BBW rat showing marked invasion by mononuclear cells. Onset of diabetes was 55 days earlier. HE x 125.

Fig. A.3 Periductal eosinophilic infiltrate in the pancreas of a 280-day-old BBW rat. HE x 125.

Fig. A.4 Pancreatic granuloma in a 385-day-old BBW rat. An islet in the upper left corner is surrounded by a predominantly lymphocytic infiltrate. Granuloma is also surrounded by lymphocytes. HE x 125.

islets were seen in young diabetic rats (Fig. 6). Islets from chronically diabetic BBW rats were generally small, decreased in number, and composed almost entirely of non-beta cells (Seemayer et al., 1982) (Fig. 7).

DISCUSSION

A wide variety of exocrine pancreatic lesions has been reported in human diabetics, but the significance of most of these remains obscure. Fibrosis, often with some degree of chronic-interstitial inflammation, is the most common finding, occurring in 50 to 60 per cent of necropsy cases (Warren, LeCompte and Legg, 1966; Kothare, 1974). Pancreatitis, although an uncommon cause of diabetes, is seen in approximately 5 per cent of diabetic patients at necropsy (Goto, Sato and Masuda, 1974; Parson, MacDonald and Shaper, 1968). Other frequent findings include fatty infiltration and arteriosclerosis of the pancreas (Warren et al., 1966).

The types of pancreatic exocrine lesions in the BBW rat (Table 1) are
PANCREATIC LESIONS IN DIABETIC RATS

Fig. A.5 Islet from a 137-day-old BBWd rat showing islet cells with pyknotic nuclei and acidophilic cytoplasm and intact islet cells. No leukocytic infiltrates were seen in any islets in this rat. Onset of diabetes was 47 days earlier. HE x 200.

Fig. A.6 One of many hyperplastic islets in a 178-day-old BBWd rat showing a peri-insular mononuclear infiltrate. Onset of hyperglycaemia was 60 days earlier. HE x 50.

Fig. A.7 Atypical small end-stage islet consisting of only a few cells, and without evidence of necrosis. HE x 200.

generally similar to those in human diabetics. Chronic interstitial inflammation is common in BBWd rats but usually lacks the prominent fibrosis seen in man. However, it is equally common in non-diabetic BBW and Wistar rats, and is also common in many other strains of laboratory rats (Kendrey and Roe, 1969). Acute or chronic pancreatitis was most common in BBWd rats (z = 1.34, P = 0.10) occurring in 10 BBWd (8.3 per cent), 1 BBWnd (2.3 per cent), and in none of the Wistar rats.

Pancreatic granulomatous lesions were seen in approximately 2 per cent of the BBWd and BBWnd rats but were not present in Wistar rats. In several instances, the granulomas were in close approximation with insulitis. Granulomas were also common in other organs (Wright et al., 1980; Wright et al., 1983a). The occurrence of spontaneous granulomatous lesions in the absence of infectious organism is consistent with the findings of others which suggest that the BBW rat has an abnormal cellular immune system (Jackson, Rassi, Crump, Haynes and Eisenbarth, 1981; Like, Rossini, Bugerski, Appel and Williams, 1979; Like, Kislanski, Williams and Rossini, 1982). Eosinophils were often present in the periphery of the granulomatous lesions. Indeed, the
presence of an eosinophilic component in both the acute and the chronic pancreatic lesions in BBW rats was often striking. This is interesting in the light of several previous studies which show that (1) infiltrates of eosinophils are common in other organs of BBWd rats (Wright et al., 1980; Wright et al., 1983a), (2) marked eosinophilia (in the absence of parasitism) is frequently seen in the peripheral blood of BBWd and BBWnd rats but does not occur in the outbred Wistar line from which BBW rats were derived (Wright, Yates, Shah, Neff, Covey and Thibert, 1983b) and (3) small numbers of eosinophils are occasionally present in the infiltrates in insulitis in BBW rats (Nakhooda et al., 1977). No eosinophilic infiltrates were observed in any of the Wistar rats. The significance of eosinophilia in BBW rats is not known but is consistent with hypersensitivity to some unknown allergen or auto-antigen.

No endocrine or exocrine pancreatic tumours were observed in this study, and are very rare in all strains of rats (Altman and Goodman, 1979). Lymphomas are relatively common in BBW rats (Kalant and Seemayer, 1979) and lymphomatous infiltration of the pancreas was seen in two rats in our series (Wright et al., 1983a).

Insulitis is a lymphocytic infiltration selectively involving the pancreatic islets. It is frequently observed in some but not all islets of JOD patients who die within one year of clinical onset (Gepts, 1977). In most cases, only small lymphocytes are seen, but the presence of macrophages, plasma cells, eosinophils, and neutrophils has also been reported (Wellmann and Volk, 1980).

BBW rat islets typically show 3 general patterns: (1) "end-stage" islets in chronically diabetic rats are small, decreased in number, composed almost entirely of non-beta cells and a few granulated beta cells; (2) mononuclear cell insulitis is usually seen at the onset of diabetes and in the subsequent few weeks; (3) normal islet histology is typical of pre-diabetic and non-diabetic BBW rats (Nakhooda et al., 1978). This picture is confirmed by our study and that of others (Seemayer et al., 1982). Most of our BBWd rats, regardless of the severity of diabetes, had at least a few typical small "end-stage" islets. These had numerous cells with acidophilic cytoplasm and pyknotic nuclei. Occasionally, hyperplastic islets suggesting functional hyperactivity were seen in the pancreas of young diabetics. Most of these had died shortly after the onset of glycosuria, but some had been diabetic for several months. One of the latter also had insulitis.

Seemayer et al. (1982) found an orderly step-wise progression in the development of insular lesions which closely correlated with the onset of diabetes. They observed insulitis in none of four 50-day-old and 5 of seven 65-day-old BBWnd ("young normoglycemic") rats. This suggests that the destructive process in the islets starts in advance of the clinical syndrome, but it has not been definitely established that glycosuria is always preceded by insulitis. They observed severe insulitis in "early" diabetic rats (1 to 3 days post-detection) but only end-stage islets and a few residual mononuclear cells in "unstable" and "stable" (7 to 22 and 41 to 63 days post-detection, respectively) BBWd rats. In contrast, our study reveals that insulitis may be quite prominent several months after the onset of glycosuria. A possible
Pancreatic lesions in diabetic rats

Explanation for the difference between these 2 studies is that the rat populations were different. Seemayer et al. (1982) utilized small numbers of a relatively young population of rats over narrow age ranges for each group. All were the offspring of only 2 breeding pairs. Our rats were all from the same colony but most were not littermates and there was a much wider range of ages at the onset of diabetes. To determine whether age of onset had any correlation with the persistence of insulitis, we examined all 45 rats that died or were killed within 70 days of onset of glycosuria (the maximal duration of diabetes in this study at which a rat was found to have severe insulitis). The mean age of onset of glycosuria in rats with persistent insulitis was 153.1 ± 71.0 days, and in those without persistent insulitis was 129.8 ± 58.7 days. There is no statistically significant difference in the ages of these groups \( F(1,18) = 0.62, \text{n.s.} \).

Insulitis was observed in 16.3% per cent of our BBWnd rats. Nakhooda et al. (1978) also reported an incidence ranging from 17 to 25 percent in BBWnd rats. This indicates that insulitis does not always cause the immediate onset of clinically apparent diabetes mellitus. However, some rats may have progressive insular involvement which in time could lead to progressively more severe hypoinsulinaemia. Indeed, many older BBWnd rats do eventually convert into BBWd rats (Wright et al., 1983a).

Summary

Pancreatic histopathology was studied in 121 BBWd, 43 BBWnd, and 33 Wistar rats. Insulitis was the most common inflammatory lesion in both BBW and BBWnd rats. The incidence was inversely associated with age and with duration of diabetes in BBWd rats, but there was no age-related pattern in BBWnd rats. Small end-stage islets were typical of BBWd rats but were not seen in BBWnd rats. Several BBWd rats showed hyperplastic islets months after the onset of diabetes, a pattern that is also seen in a small percentage of human JOD patients. Several non-specific exocrine inflammatory lesions occurred in both BBWd and BBWnd rats: acute and/or chronic pancreatitis, eosinophilic infiltrates, granulomatous lesions and acute and/or chronic interstitial inflammation. Only chronic interstitial inflammation was seen in outbred Wistar rats.

Acknowledgments

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Spontaneous Thyroiditis in BB Wistar Diabetic Rats

J. R. Wright, Jr., D. A. Senhauser, A. J. Yates, H. M. Sharma, and P. Thibert

Department of Pathology, Ohio State University College of Medicine, Columbus, Ohio, and Animal Resources Division, Sir Frederick G. Banting Research Centre, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada

Abstract. Spontaneous lymphocytic thyroiditis was observed at necropsy in 36 BB Wistar diabetic rats (63.2%) and in eight of their nondiabetic siblings (42.1%). The incidence of thyroiditis decreased both with age and with longer duration of diabetes. All rats with pancreatic insulitis (a manifestation of the onset of diabetes) also had thyroiditis. BB Wistar rats with insulitis had more severe lymphocytic thyroiditis, characterized by lymphocytic, plasmacytic, and macrophage infiltration of thyroid interstitium and follicles. A milder, mostly perivascular and interstitial lymphocytic thyroiditis was characteristic of lesions in rats which did not have insulitis. The histological appearance of the thyroiditis suggests that these rats may be subject to autoimmune disease at the onset of diabetes which involves sites other than just the pancreas.

Although the exact mechanism(s) responsible for the onset of human juvenile-onset diabetes is unknown, cell-mediated immunity probably is involved [2, 21]. This is supported by the occurrence of a mononuclear cell insulitis that selectively destroys the insulin-producing beta cells in the pancreatic islets concurrent with the onset of the diabetic syndrome in both the BB Wistar rat, a promising new animal model for human juvenile onset diabetes [19, 20], and man [8, 34]. The BB Wistar diabetic syndrome occurs in the absence of obesity and is characterized by hyperglycemia, glycosuria, ketoacidosis, insulinopenia, glucagonemia, and hyperlipemia [19, 20, 22]. Spontaneous diabetes occurs in 30 to 55% of the offspring of diabetic matings. Onset is abrupt with rapid weight loss due to dehydration as a result of marked polyuria. In the absence of insulin therapy, death usually follows the onset of symptoms by one to two weeks.

Although the pancreas of the BB Wistar rat has been studied extensively, relatively little is known about spontaneous diseases in other organs of this animal, therefore, we conducted an extensive necropsy study. Our results and the results of others have shown that the BB Wistar rat also develops a wide variety of inflammatory, degenerative, neoplastic, and developmental disorders [14, 29, 36–41]. A significant proportion of these rats develop spontaneous lymphocytic thyroiditis—a finding that recently has been reported by another group [30].

To
Spontaneous Thyroiditis in BBW Rats

<table>
<thead>
<tr>
<th>Grade of interstitial infiltrate</th>
<th>Follicular involvement</th>
<th>Bilateral lesions</th>
<th>Infiltrate cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 1 2 3</td>
<td>L PC M All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 22 9 5</td>
<td>10</td>
<td>21^2</td>
<td>36 11 12 7</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11 7 10</td>
<td>1</td>
<td>4</td>
<td>8 4 1 1</td>
</tr>
</tbody>
</table>

1 Each value represents the number of rats in that category. Thyroids were examined in 57 diabetic and 19 nondiabetic rats. Histological evidence of thyroiditis was found in 36 diabetic and nine nondiabetic rats.

2 Only one lobe of the thyroid was examined in three rats.

3 L = lymphocytes; PC = plasma cells; M = macrophages, All = all three cell types present.

date there has been no histological description of this entity, nor has the incidence of thyroiditis been examined in relation to the age of the rats.

Materials and Methods

A breeding colony of BB Wistar rats was established in the Pathology Department at The Ohio State University from the parent colony at the Health Protection Branch of Health and Welfare of Canada. Detailed description of our animal housing facilities and animal husbandry techniques has been reported previously [40]. Complete necropsies were done on 57 diabetic rats and 19 of their nondiabetic siblings which either died spontaneously or were sacrificed for experimentation. All major organs were examined grossly, fixed in neutral phosphate buffered formalin, and processed for light microscopy. Tissue sections were stained with hematoxylin and eosin (HE). At least four sections from different parts of the thyroid were examined in each rat. Thyroids were graded as either normal or one of three grades of severity of thyroiditis based on the extent of interstitial infiltrate. The presence of follicular involvement was noted when present. Lesions were determined to be either bilateral or unilateral—lesions involving only the isthmus were considered unilateral. The presence of ultimobranchial duct cysts also was tabulated. All statistical analyses were by chi-square test, binomial test, or one-tailed z-test.

Results

Lymphocytic thyroiditis was observed in 36 diabetic rats (63.2%) and in eight nondiabetic rats (42.1%). Inflammatory lesions were bilateral in two-thirds of the diabetic rats and one-half of the nondiabetic rats (table I). Most of the lesions were characterized as predominantly perivascular, mild focal, or multifocal interstitial lymphocytic thyroiditis (grade 1). The most severe lesions (grade 3) were multifocal and involved over 25% of the interstitium. Those lesions intermediate in severity between grades 1 and 3 were classified as grade 2. The higher grades were less frequent and were characterized by extensive multifocal inflammatory infiltrates (fig. 1) frequently consisting of lymphocytes, plasma cells, and macrophages (fig. 2, 3). Follicular invasion was more common in these lesions (fig. 4). The latter type of lesion was characteristic of BB Wistar rats with insulitis. It is interesting that nine rats (seven diabetic and two nondiabetic) had insulitis, and all nine also had
Fig. B.1 Extensive multifocal mononuclear cell infiltrate in BB Wistar rat thyroid tissue. Infiltrate is predominantly interstitial and perivascular. HE.

Fig. B.2 Large numbers of plasma cells and plasmacytoid lymphocytes composing some of the infiltrates. Infiltrate is in close association with thyroid follicles with some disruption of follicular basement membrane. HE.

Fig. B.3 Mixed chronic inflammatory infiltrate adjacent to several thyroid follicles.

Fig. B.4 Extensive follicular involvement by inflammatory infiltrate with apparent disruption of follicular architecture and dissolution of thyroid epithelial cells in the most severely affected thyroids. HE.
Table B.2 Incidence of lymphocytic thyroiditis in BB Wistar rats by age and by duration of diabetes

<table>
<thead>
<tr>
<th>Age or duration of diabetes in days</th>
<th>0-120</th>
<th>121-240</th>
<th>241-360</th>
<th>360-480</th>
<th>481-800</th>
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<td>Diabetic</td>
<td>Age</td>
<td>2/2 (100%)</td>
<td>14/20 (70%)</td>
<td>17/27 (63.0%)</td>
<td>3/8 (37.5%)</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>19/27 (70.4%)</td>
<td>13/21 (61.9%)</td>
<td>4/9 (44.4%)</td>
<td>—</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>Age</td>
<td>1/2 (50.0%)</td>
<td>0/2</td>
<td>1/2 (50.0%)</td>
<td>3/6 (50%)</td>
</tr>
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</table>
thyroiditis (binomial test, \( p = 0.002 \)). Furthermore, the severity of the thyroiditis, as manifested by the extent of interstitial involvement (\( X^2 = 43.19, \text{df} = 3, p < 0.001 \)) and follicular involvement (\( X^2 = 16.73, \text{df} = 1, p < 0.001 \)), was much greater in rats with insulinitis. Rats with insulinitis also had multiple inflammatory cell types present in the thyroid lesion more frequently (\( X^2 = 20.57, \text{df} = 2, p < 0.001 \)). Table II shows that the incidence of thyroiditis in diabetic rats decreases with age and with duration of diabetes, but this relationship was not statistically significant since most of the rats were in the two middle age groups. No age pattern was apparent in nondiabetic rats.

Ultimobranchial duct cysts were observed in 13 diabetic (22.8%) and three nondiabetic (15.8%) rats. The margins of these cysts had a prominent chronic inflammatory infiltrate in six of the diabetic rats.

**Discussion**

The histologic appearance of thyroiditis in BB Wistar rats is consistent with the features of autoimmune thyroiditis described in man [26, 32]. Although occasional parenchymal cells were enlarged and had eosinophilic cytoplasm, no unequivocal Askanazy cells were seen. Although Askanazy cells are a prominent feature in the adult form of Hashimoto's thyroiditis in man, they usually are not seen in all forms of human autoimmune thyroiditis [32] nor are they a prominent feature of experimentally induced or spontaneous autoimmune thyroiditis in other rat studies [10, 23].

Although the BB Wistar rat is very susceptible to infection [36, 38, 41], an infectious etiology for the thyroiditis seems unlikely because of the absence of acute thyroiditis, suppuration, or subacute thyroiditis, features associated with infectious thyroiditis [32], and the absence of involvement of adjacent tissues. Furthermore, the rats were housed in a semi-barrier housing facility to minimize exposure to infectious agents. No thyroid lesions (except cysts) were seen in outbred Wistar rats kept in the same facility.

Although spontaneous autoimmune thyroiditis is not rare in man, there are few naturally occurring animal models [4, 24]. Inbred strains of laboratory animals which develop lymphocytic thyroiditis include Obese Strain chickens [35], laboratory pure-bred beagles [9], marmosets of the genus *Callithrix* [16], and the Buffalo strain rat [10]. Although the latter is the only strain of the rat which develops spontaneous thyroiditis, it has been induced in rats by several means of immunosuppression including chemical agents [15, 28], thymectomy [23, 28], and irradiation [23]. This is particularly relevant because of findings showing that the BB Wistar rat is naturally immunocompromised [13, 38].

In an earlier study, a 59% incidence of lymphocytic thyroiditis in BB Wistar diabetic rats and an 11% incidence in their nondiabetic siblings was reported [30], but the functional significance of this lesion is uncertain since serum \( T_3 \) and \( T_4 \) levels were normal in rats with thyroiditis. In a second study, the effect of iodide supplementation on the incidence of thyroiditis in BB Wistar rats was examined
Spontaneous Thyroiditis in BBW Rats

[31]. There was a slightly higher incidence (not statistically significant) of lymphocytic thyroiditis in diabetic and nondiabetic rats receiving tap water with iodide than in those receiving tap water without iodide supplementation.

There are several pertinent facts concerning the pattern of occurrence of thyroiditis in BB Wistar rats that are compatible with an autoimmune pathogenetic mechanism. First, the incidence is slightly higher (not statistically significant) in female than male diabetic (71.4% to 62.0%) and nondiabetic (50.0% to 28.6%) rats. It is well established that women have a higher prevalence of Hashimoto's thyroiditis as well as most other autoimmune diseases [24]. Second, the incidence is higher in diabetic than nondiabetic rats ($Z = 1.61, p = .05$), a finding consistent with the earlier study [30]. This is interesting because several studies have reported an association between juvenile-onset diabetes and autoimmune thyroid disease [12, 21]. Relevant to this is the observation that HLA-B8 and DR3 histocompatibility antigens occur with a greater frequency in patients with insulin-dependent diabetes, Graves' disease, and Hashimoto's thyroiditis [2, 3, 18]. This pattern is characteristic of a small subgroup of the human juvenile-onset diabetic population and has been termed "the syndrome of polyendocrine autoimmunity" [1, 2, 5]. No lymphocytic thyroiditis has been reported in other strains of diabetic rodents in which the thyroid has been examined [27, 33]. Third, there is a higher incidence of thyroiditis in the younger rats (table II)—this also is true for insulitis. (Insulitis in BB Wistar rats occurs at the onset of diabetes, typically between 60 and 160 days of age). Finally, nine BB Wistar rats (seven diabetic and two nondiabetic) in this necropsy series had insulitis and all nine also had thyroiditis. Therefore, it seems likely that the onset of diabetes in BB Wistar rats is not only associated with a mononuclear cell insulitis, but also a mononuclear cell thyroiditis. Since the insulitis in BB Wistar rats is transient [19, 20], it was only seen in rats dying soon after onset of diabetes. On the other hand, data in table II show that the thyroiditis may be present for up to a year after the onset of diabetes. This is in contrast to the incidence of human autoimmune thyroiditis which increases with age [24].

It is well established that there is a tendency for more than one autoimmune disorder to occur in the same patient. When this happens, all of the autoimmune disorders tend either to be very organ specific as in Hashimoto's thyroiditis or systemic with no organ specificity as in systemic lupus erythematosus [25]. Autoimmune disorders associated with juvenile-onset diabetes are of the organ specific type [11].

Results of several studies suggest that both the insulitis and thyroiditis in BB Wistar rats may be autoimmune in nature. Serum immunofluorescence studies have not demonstrated circulating autoantibodies to pancreatic islet cells in BB Wistar rats [6, 7]. However, antibodies against the islet cell plasma membrane have been detected by radioligand assay in 12 of 14 (85%) BB Wistar diabetic rats studied three to eleven days after onset of hyperglycemia [6]. Antithyroglobulin autoantibodies have been found by serum immunofluorescence in BB Wistar rats. A second study reported no antithyroid microsomal antibodies but did demonstrate
antibodies to thyroid colloid antigens in 5% to 17.9% of the BB Wistar rats studied [7]. Another study reported thyroid colloid autoantibodies in 34 of 48 diabetic (71%) and eight of 39 nondiabetic BB Wistar rats. In addition, they found evidence of autoantibodies to thyroid follicular cells in some rats [17]. In spite of these results, there is still insufficient evidence to determine the specific pathogenetic mechanism responsible for thyroiditis and insulitis in BB Wistar rats. The concurrence of these lesions in the same rats suggests that these mechanisms are related closely.

Acknowledgements

This work was supported by the Department of Pathology, the College of Medicine, and the Graduate School of The Ohio State University; the Upjohn Company; and N.I.H. grant NS-18026. Mr. James R. Wright, Jr. was supported by the Samuel J. Roessler Research Scholarship. The authors also would like to thank Racchel Tigncr for her statistical assistance and Dave Covey and Bruce Thompson for their excellent maintenance of the BB Wistar colonies in Columbus and Ottawa.

This work was presented in part at the Landacrc Society's 25th Anniversary Medical Student Scientific Session, Columbus, Ohio, March 19, 1981.

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Appendix C
Testicular Atrophy in the Spontaneously Diabetic BB Wistar Rat

JAMES R. WRIGHT, JR., BS,
ALLAN J. YATES, MD, PHD,
HARI M. SHARMA, MD, FRCP(C),
CHULL SHIM, MD, RAECHL L. TIGNER, AB,
and PIERRE THIBERT, DVM

Complete gross and microscopic postmortem examinations were performed on 100 BB Wistar diabetic rats, 27 BB Wistar nondiabetic siblings, and 41 Wistar rats, and the incidence of testicular lesions was tabulated. Testicular atrophy was the predominant finding in all three groups of rats, but atrophy occurred at a much younger age in the diabetic rats. There was a strong relationship between the duration of diabetes and the presence of atrophy, which was stronger than the relationship between age and atrophy. The testicular atrophy observed in the diabetic rats was morphologically similar to the senile testicular atrophy in the nondiabetic rats. Histologic findings that were associated with increasing severity of atrophy were multinucleated giant cells in the lumens of seminiferous tubules, increased interstitial connective tissue, Leydig cell hyperplasia, and thickening of the tunica albuginea. Testicular atrophy has also been reported in human diabetics. Therefore, the BB Wistar rat may be a useful model for investigating this aspect of diabetes mellitus. (Am J Pathol 1982, 108:72-79)

THE BB WISTAR (BBW) rat is an outbred strain of spontaneously diabetic rats that originated in the Bio Breeding Laboratories Ltd. of Ottawa, Canada. The BBW rat is a promising model for insulin-dependent diabetes because it spontaneously develops beta cell destruction with subsequent insulinopenia, hyperglycemia, hyperglucagonemia, glycosuria, ketocidosis, and hyperlipemia. However, except for the pancreatic lesions, very little is known about other spontaneous diseases that occur. Therefore, we conducted a necropsy study to determine the types and frequencies of pathologic abnormalities in our breeding colony of BB rats. The most frequent abnormality in the male rats was testicular atrophy. Although testicular atrophy has been reported in both human diabetics and in a wide variety of diabetic animals, there are few reports of systematic detailed studies of this abnormality.

Materials and Methods

A total of 100 BB Wistar diabetic rats (BBWd), 27 BB Wistar nondiabetic siblings (BBWnd), and 41 standard Wistar (w) rats with mean ages of 254 ± 100 days, 241 ± 122 days, and 281 ± 162 days, respectively, were examined. Because of the extreme susceptibility of BB Wistar rats to infection, all rats were maintained in a semibarrier housing facility. The details of animal maintenance and care have been reported previously. Every day all animals were weighed, and the urine of diabetic rats was examined for ketones and glucose with the use of Ketostix (Ames Co., Elkhart, Ind) and Testape (Eli Lilly, Indianapolis, Ind), respectively. We administered protamine zinc (U-40) insulin (Eli Lilly) subcutaneously on a daily basis to maintain 4+ glycosuria without ketonuria.

Supported in part by the Department of Pathology, College of Medicine, and the Graduate School of The Ohio State University, the Upjohn Company, and NIH Grant NS-18026. Mr. Wright was supported by a Samuel J. Roesler Research Scholarship. Presented in part at the American Association for Laboratory Animal Science Meeting, September 22, 1981, Salt Lake City, Utah. Accepted for publication, February 24, 1982. Address reprint requests to Allan J. Yates, MD, PhD, Neuropathology Unit, Department of Pathology, The Ohio State University, 105 Upham Hall, 473 West 12th Avenue, Columbus, OH 43210.
Necropsy was performed on all rats that died spontaneously or were sacrificed for experimentation either immediately or following storage at 4°C, usually for no longer than 12 hours after death. Thoracic and abdominal organs were removed en bloc; and, following dissection, individual organs were weighed, fixed in neutral phosphate-buffered formalin, and processed for light microscopy. Tissue sections were routinely stained with hematoxylin and eosin. Tissue and fluid specimens suspected of antemortem bacterial infection were placed in disposable collection tubes and cultured for aerobic and anaerobic microorganisms. Paraffin sections of such tissues were stained with Gram's stain, acid fast blue, and Gomori's methenamine silver for bacteria and fungi.

In addition to determining testicular mass and body mass, we determined the presence and distribution of testicular atrophy (unilateral and bilateral) histologically. Testes from all three groups of rats were rated as normal, hypocellular, or atrophic. Hypocellular testes had seminiferous tubules with decreased cellularity, but all retained some germ cells. Atrophic testes were those that had seminiferous tubules without any germ cells. We classified these as Grades 1 or 2 on the basis of the extent of atrophy. Testes were considered to have Grade 1 (minimal) atrophy if there were fewer than 3 atrophic tubules per cross-section; testes with Grade 2 (severe) atrophy were those with more than 5 atrophic tubules per cross-section. We used chi-square analysis to compare these categories with a variety of specific histologic abnormalities, including the presence of multinucleated giant cells in the lumens of tubules, increased amounts of interstitial tissue, increased density of Leydig cells, and increased thickness of tunica albuginea. All of these were rated subjectively as normal or as having one of three grades of severity and related to the ages of the animals by the chi-square test. Since pneumonia is known to affect spermatogenesis severely,† the presence or absence of pneumonia was also noted. The duration of diabetes in days for each BBWd rat was taken from its treatment records.

Results

Figure 1 shows the distribution and severity of testicular lesions according to the ages of the rats. All three types of rats (BBWd, BBWnd, and W) developed minimal and severe testicular atrophy. One-third of BBWd rats in the youngest age group had hypocellular testes. Both minimal testicular atrophy and hypocellularity occurred in the 121-240-day-old age group of BBWd, and the frequency and severity of the lesions progressively increased with age in these rats. Similar changes occurred in the BBWnd rats, but at an older age and with less severity. Testes from Wistar rats showed no degenerative testicular changes until the second oldest age group, but all rats had severely atrophic testes in the 481-600-day-old age group. The relationship between the age of the rats and the degree of atrophy was examined by a one-way analysis of variance (unweighted means solution). Table I shows that the severity of atrophy increased significantly ($P < 0.001$) with age in both BBWd and W rats. The same trend is seen in the BBWnd rats but is not statistically significant, because only 3 of these rats developed atrophy. The presence of atrophy related more strongly to dura-
Table C.1 Distribution of Age and Duration of Diabetes by Degree of Testicular Atrophy

<table>
<thead>
<tr>
<th>Rat type</th>
<th>Days</th>
<th>Mean ± SEM</th>
<th>Statistical relationship to degree of atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBWd</td>
<td>0-120</td>
<td>254 ± 10.0</td>
<td>F(2, 97) = 21.25, P &lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>6</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>49</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>BBWnd</td>
<td>121-240</td>
<td>147 ± 10.0</td>
<td>F(2, 97) = 38.29, P &lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>241-360</td>
<td>240 ± 23.6</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>361-480</td>
<td>280 ± 30.2</td>
<td>F(2, 26) = 17.56, P &lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*Values are numbers of rats for which precise ages and duration of diabetes are known.
†Statistical analysis by one-way analysis of variance using the unweighted means solution. NS = not statistically significant.

Table C.2 Body and Testicular Weights

<table>
<thead>
<tr>
<th>Body mass (grams)</th>
<th>Testicular weights ± SEM (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>BBWd 329.8 ± 8.5</td>
<td>2.88 ± 0.06</td>
</tr>
<tr>
<td>n = 87</td>
<td>n = 56</td>
</tr>
<tr>
<td>F (1, 83) = 42.81, P &lt; 0.001</td>
<td>F (2, 83) = 88.79, P &lt; 0.001</td>
</tr>
<tr>
<td>BBWnd 301.9 ± 26.3</td>
<td>3.13 ± 0.15</td>
</tr>
<tr>
<td>n = 19</td>
<td>n = 17</td>
</tr>
<tr>
<td>F (1, 15) = 43.05, P &lt; 0.001</td>
<td>F (2, 15) = 43.05, P &lt; 0.001</td>
</tr>
<tr>
<td>Wistar 449.7 ± 27.8</td>
<td>3.56 ± 0.10</td>
</tr>
<tr>
<td>n = 33</td>
<td>n = 26</td>
</tr>
<tr>
<td>F (1, 29) = 40.64, P &lt; 0.001</td>
<td>F (2, 29) = 36.36, P &lt; 0.001</td>
</tr>
</tbody>
</table>

*Each measure is the combined mass of both testes from each rat. SEM = standard error of the mean.
†Statistical analysis examining testicular weight with respect to histologic severity of atrophy was by analysis of covariance with body weight as covariate. Includes only rats for which both body mass and testicular mass were known.
‡Correlations between testicular weight and body weight were 0.376, 0.796, and 0.534 for BBWd, BBWnd, and Wistar rats, respectively.
Discussion

Structural changes in the testes of human diabetics were first observed in 1878 by Paschutin and later substantiated by others. Several of these investigators attempted to relate these histologic abnormalities to sexual impotence, a disorder present in approximately 50% of diabetic men of reproductive age. A study by Singhal et al suggests that testicular abnormalities are present in both impotent and sexually potent diabetics with similar frequency. Impotence is presently believed to be due to diabetic peripheral neuropathy and unrelated to these testicular changes.

Although the histologic changes in the testes have been studied extensively in chemically induced diabetes, they have been examined in only two other strains of spontaneously diabetic animals, the obese-hyperglycemia AO mouse and the Chinese hamster. Both studies described hypocellularity or maturation arrest as the predominant finding, rather than a total absence of germ cells within seminiferous tubules. In addition to these milder changes, histologic changes observed in the BB Wistar rat include both mild and severe atrophy. Furthermore, we have examined these changes with respect to both age and duration of diabetes and have simultaneously compared them with the senile testicular atrophy that occurs in nondiabetic rats.

Since testicular atrophy is prevalent in most strains of aged rats, age was considered a likely contributing factor to the high incidence of testicular lesions in the BBW Wistar rats. Burck reported that

![Fig. C.2 Transected rat testes. Normal Wistar rat on the left and BB Wistar diabetic rat with bilateral atrophy on the right. Line is 1 cm.](image)

<table>
<thead>
<tr>
<th>Table C.3 Numbers of Rats With Different Degrees of Severity of Testicular Histologic Abnormalities and Their Relationship to Degree of Atrophy and Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat type</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>BBWd</td>
</tr>
<tr>
<td>BBWd</td>
</tr>
<tr>
<td>Wistar</td>
</tr>
<tr>
<td>BBWd</td>
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<td>BBWd</td>
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<td>Wistar</td>
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<tr>
<td>BBWd</td>
</tr>
<tr>
<td>BBWd</td>
</tr>
<tr>
<td>Wistar</td>
</tr>
</tbody>
</table>

*All statistical analyses by chi-square test. Chi-square values and degrees of freedom printed below P values.
at least limited atrophy was present in all rats over 18 months of age and was usually severe in rats older than 24 months. He found that atrophic testes of old rats frequently had Leydig cell hyperplasia, interstitial edema, and tubules with multinucleated giant cells or only Sertoli cells. The changes described by Burek were consistent with the histologic changes observed in all three types of rats in our study. Several investigators have frequently reported benign or malignant tumors associated with testicular atrophy in senile rats, but these were not seen in any rats in our study. This is probably due to the greater age of their animals. In our study, testicular atrophy was first observed in BBW and W rats at 355 and 361 days of age, respectively; the earliest age of onset in the BBW diabetic rats was 148 days. Figure 1 shows clearly that the incidence of atrophy increased with age in all rats but occurred at a much younger age in BBW rats. Saksena et al. reported that sperm production in Sprague-Dawley rats remained maximal from 72 days of age to beyond 450 days of age. This appears consistent with our histologic findings in standard Wistar rats but not in the BBW rats. Age is undoubtedly a very important factor in determining the presence of atrophy in all groups of rats, but the duration of diabetes is a much better predictor of atrophy in BBW than is age (Table 1).

The severity of testicular lesions in diabetic men and animals varies widely from study to study. Several have reported only a hypocellularity of germinal cells, others more severe atrophy, and some only Sertoli cells present. This discrepancy may possibly be explained by the severity of the diabetic state in these different studies. Several investigators have suggested that testicular atrophy is more frequent in poorly controlled diabetics.

Several studies on diabetic men and rats have reported increased interstitial tissue in atrophic testes. Only Schofield et al. reported no increase in interstitial tissue. We observed a slightly significant increase in interstitial tissue in rats with testicular atrophy, regardless of the presence or absence of diabetes. We therefore feel that increased interstitial tissue is probably a general feature of testicular atrophy and is not specifically related to diabetes.

In all three groups of rats, multinucleated giant cells (Fig. 3) were frequently seen in the lumens of degenerating seminiferous tubules. Other studies have reported similar findings in both senile and diabetic rats. These giant cells are believed to be fused spermatids. To our knowledge, multinucleated giant cells have not been reported in the seminiferous tubules of man.

Several studies utilizing either rats with chemically induced diabetic or genetically obese (insulin-resistant) mice have reported a decrease in the number of Leydig cells. It is possible that the diabetogenic chemicals used by some have a toxic effect on the Leydig cells, because alloxan is known to have severe toxic effects on many organ systems, including the testes. Leydig cell hyperplasia was observed in this study and in a study by Rosenmann et al. Both his
study and ours utilized rats that were not obese and did not have chemically induced diabetes. Interestingly, Ayud has reported Leydig cell hyperplasia in a series of 30 diabetic men. We also observed Leydig cell hyperplasia in non-diabetic rats with senile testicular atrophy. This is consistent with senile atrophy in non-diabetic rat strains.

Maturation arrest within seminiferous tubules has been reported in a variety of studies of human and animal diabetics. Most of these investigators have attributed this to hyposecretion of gonadotropic hormones. This explanation seems unlikely, because several recent studies have shown that testosterone levels and gonadotropin levels are normal in diabetic men, but an endocrinologic mechanism is suggested by the observation that pancreatectomy or alloxan diabetes not only affects the germinal epithelium but also suppresses the development of secondary sexual characteristics in animals.

Some investigators have attributed testicular lesions in diabetics to diffusion or perfusion problems caused by basement membrane thickening of tunica propria, microangiopathy, or atherosclerosis. Since seminiferous tubules are avascular, diffusion from the interstitial vessels through the tunica propria is necessary to nourish the germ cells and Sertoli cells in each tubule. Only a few studies have reported basement membrane thickening, and others have reported its absence. In the present study, microangiopathy and atherosclerosis were not observed by light microscopy and so could not be involved in the pathogenesis of the lesions. Only a few rats had tubules with marked basement membrane thickening, and this thickening may have been secondary to the atrophy rather than causative.

Spermatogenesis in man and the rat is generally similar, except that it proceeds in waves in the rat. In both, proliferation of the germinal epithelium is controlled by a complex milieu of hormones and other factors. The number of spermatozoa produced depends on temperature, available lighting, nutritional factors, age, pituitary gonadotropin levels, testosterone levels, and other variables. It is unlikely that temperature or lighting played a significant role in the incidence of testicular atrophy, because the housing facility was consistently maintained at 72 F with alternating 12-hour periods of artificial light and darkness.

In summary, a high incidence of testicular abnormalities characterized by a partial or total loss of germinal epithelium, frequently with a relative sparing of Sertoli cells, was observed in BB Wistar diabetic rats, their non-diabetic siblings, and standard Wistar rats. These lesions occurred at a much younger age in the diabetic rats than in either the BBWnd or W rats. Similar findings have been reported in human diabetics as well as other animal models for diabetes.
though atrophic testes of diabetic animals are histologically similar to those with senile atrophy, the exact etiology and pathogenesis of these lesions remain to be elucidated. The BB Wistar rat appears to be an excellent model for study of the pathogenetic mechanisms involved. Furthermore, the significance of these findings to investigators attempting to breed BB Wistar rats for other types of studies is reflected in the lower rate of breeding success in BBWd rats.

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TESTICULAR ATROPHY IN DIABETES

28. Ayau H: Discussion of hypogonadism in male diabetic subjects, p 520

Acknowledgments

The authors wish to thank Dave Covey and Bruce Thompson for the excellent maintenance of the BB Wistar breeding colonies at Columbus and Ottawa, respectively; Dr. Leona Ayers for microbiological studies; and Drs. Charles C. Capen and Henry A. Wise II for critically reviewing the manuscript.
Appendix D
Atrophy, Testis, Rat
James R. Wright, Jr.

**Synonyms.** Testicular degeneration, gonadal degeneration.

**Gross Appearance.** Severely atrophic testes are small, soft, and covered by a smooth but wrinkled, rubbery, yellow-tan tunica albuginiae. The testicular mass may be decreased by more than 50%. (Normal testes in a 500-g rat weigh approximately 2–2.25 g each.) Upon cutting through the tunica albuginiae, a scant amount of edema fluid may be released. The seminiferous tubules are loosely held together. In very old rats of some strains (particularly the Fisher 344 strain), small gray and white nodules may be present within the interstitial tissue. Testicular atrophy is usually bilateral, but marked unilateral atrophy can occur. Atrophic changes occur over a continuum of severity.

**Microscopic Appearance.** Testicular atrophy is an acquired decrease in testicular size from a prior normal state. Histological appearance varies depending on both etiology and duration, but several general patterns can be identified. Degenerative changes involving seminiferous tubules may be broadly categorized as complete atrophy (only Sertoli's cells remaining), hypocellularity, or maturation arrest. Complete atrophy, the most severe form of seminiferous tubular degeneration, refers to total absence of germinal epithelium and is irreversible. Only Sertoli's cells are present within these tubules (Fig. 263). The diameter of involved tubules is markedly decreased. Thickening of the tunica propria and basement membrane is often present (Fig. 264). "Sertoli-only" change may be either focal, involving only a few tubules, or diffuse. Interstitial tissue is usually increased (Fig. 265). Leydig's cell hyperplasia may be present (Fig. 266). This pattern is characteristic of senile atrophy, atrophy of chronic diabetes mellitus, chronic vitamin E deficiency, chronic zinc deficiency, and chronic cryptorchidism. 

**Hypocellularity** refers to hypoplasia of all germ cell types. Spermatogonia, spermatocytes, spermatids, and spermatozoa are present, but the numbers of each type are decreased, resulting in an overall thinning of the germinal epithelium and decreased tubular diameters (Figs. 267, 268). Because there are fewer germ cells, there will appear to be proportionally more Sertoli's cells. The interstitial tissue and Leydig's cells are generally unremarkable. This pattern appears to precede complete atrophy when atrophic changes are progressing slowly. Hypocellularity is most common in old rats and rats with diabetes of intermediate duration.

**Maturation arrest** refers to an abrupt cessation of spermatogenesis at a particular developmental stage. -

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[Fig. D.263 (upper left). Severe atrophy, testis, rat. Seminiferous tubules are lined by Sertoli's cells; germ cells are absent; tubules are decreased in diameter. The space between the tubules and interstitial tissue is an artifact. Formalin fixation causes tubular shrinkage. H & E, x 400 -

[Fig. D.264 (lower left). Atrophy, seminiferous tubules, testis, rat. Note the small distorted tubules without germinal epithelium with marked thickening of the tunica propria. Leydig's cells contain PAS-positive, acid-fast pigment. PAS, x 400 -

[Fig. D.265 (upper right). Focal atrophy, testis, 551-day-old Wistar rat. At the bottom are some normal tubules and interstitial tissue. Severely atrophic tubules and markedly increased interstitial tissue (upper). Focal dystrophic calcification (arrow). H & E, x 63 -

[Fig. D.266 (lower right). Leydig's cell hyperplasia, testis, 2-year-old Wistar rat. Nodular (A) and (B). H & E, x 400 -

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James R. Wright, Jr.


stage. A full complement of immature cells is present, but no mature forms are produced (Fig. 269). Because cell division is diminished or absent, the tubular diameter is decreased. Interstitial tissue is often unremarkable, but edema may be present. Leydig's cells are usually present in normal numbers. This pattern is characteristic of decreased gonadotrophin levels, inanition, early chemically induced diabetes mellitus, early zinc deficiency, and manganese deficiency.

The initial event common to these three histological patterns is sloughing of the more mature portions of the germinal epithelium into the tubular lumens. Often, the sloughed cells are cytologically unremarkable; however, marked necrosis is seen in vitamin E deficiency.

Multinucleated giant cells arising from sloughed germinal epithelium are common to most types of degenerative changes in the testes (Mason 1933; Cohen and de Vries 1969). They consist of aggregations of secondary spermatocytes or spermatids whose cytoplasm has fused into an eosinophilic syncytiurn. The nuclei usually appear identical to those of normal secondary spermatocytes or spermatids. However, giant cells have characteristic "crescent-like" nuclei in E-avitaminosis (Fig. 270). Multinuclear giant cells are believed to be the product of cytoplasmic fusion of adjacent cells rather than nuclear division without cytoplasmic division because of the rapidity of their formation (as early as 6 h after injury) and because evidence of mitotic activity is not seen in the multinucleated giant cells (Cohen and de Vries 1969). Bizarre hyperchromatic mononuclear giant cells have been described following thermal injury to the testis (Cohen and de Vries 1969). Sertoli's cells, interstitial macrophages, and Leydig's cells sometimes contain abundant lipofuscin pigments (Fig. 264). Atrophic seminiferous tubules may contain extracellular deposits, especially near the basement membrane. These yellow-brown pigments exhibit autofluorescence when they are examined in unstained histological sections under ultraviolet light. The pigments are typically acid-fast, negative for iron stains, and positive for PAS, oil red "O", and Sudan stains. Although lipofuscinosis is most characteristic of vitamin E deficiency (Swensen and Telford 1973; Nelson 1980), acid-fast pigments may also be found throughout the testes of aged rats.

**Ultrastructure.** The diagnosis of testicular atrophy does not require electron microscopy.

**Differential Diagnosis.** The diagnosis of testicular atrophy is relatively straightforward and is dependent upon an acquired decrease in testicular size and mass. Testicular hypoplasia is probably exceedingly rare.

Chronic degenerative changes following testicular infarction may mimic end-stage testicular atrophy. Spontaneous infarction occurs rarely but may be induced experimentally with surgery (Steinberger 1970) or toxic doses of cadmium (Gunn and Gould 1970). Cadmium appears specifically to damage the endothelium of the testicular capillaries, an effect that is prevented by simultaneous administration of zinc.

**Biological Features.** Numerous factors including temperature, available lighting, pituitary gonadotrophin levels, testosterone levels, age, and nutritional factors affect the function and integrity of the germinal epithelium or rat testes. In some strains of seasonally breeding wild rats, temporary testicular degeneration characterized by maturation arrest at the primary spermatocyte stage occurs during winter. Degenerative changes are accompanied by low levels of serum FSH, LH, and androgens. Reactivation of spermatogenesis occurs during the spring (Kerr et al. 1980; Irby et al. 1984).

At 30-40 days of age, rat testes descend into the scrotum; the inguinal canal remains open allowing the testes to retract and descend freely throughout life (Bivin et al. 1979). Spontaneous cryptorchidism occurs rarely. However, a strain with a genetic predisposition has been described (Fechheimer 1970). A high incidence has also been reported in biotin-deficient rats (Russfield 1967). On the other hand, surgically induced, experimental cryptorchidism has been extensively studied in the rat. Typical atrophic changes occur.
whether induced in prepubertal or adult rats. "Sertoli-only" change requires approximately 1 month (Davis and Firlit 1966; Leeson and Leeson 1970). Temperature elevation of the cryptorchid testis is presumed to be the insult initiating the degenerative changes.

Most rat strains reach sexual maturity at about 35 days of age (Russfield 1967). Maximal sperm production occurs from about 70 to 450 or more days of age (Saksena et al. 1979). Testicular atrophy (hypocellularity type) occurs sporadically in rats over 1 year of age and is nearly universal by 18 months of age. Sertoli-only change and markedly increased amounts of interstitial tissue (Fig. 265) are usually present in older rats (Wright et al. 1982; Burek 1978; Coleman et al. 1977; Anver and Cohen 1979). These are usually associated with Leydig's cell hyperplasia, which may be string-like or nodular (Fig. 266). The frequency of interstitial cell tumors with senile atrophy varies from 0% to 100% depending on the rat strain. In the F344 strain these interstitial cell tumors are especially frequent (Burek 1978; Coleman et al. 1977; Lutzen and Ueberberg 1973). Senile atrophy is commonly associated with polyarteritis nodosa of the testicular arteries and focal dystrophic calcification of seminiferous tubules (Fig. 265).

Inanition in the rat results in generalized weight loss and a slightly larger than proportional testicular weight loss. Acute starvation causes sloughing of the distal portions of the germinal epithelium, giant cell formation, and cessation of spermatogenesis. This is followed by maturation arrest. Chronic starvation is characterized by small tubules lined by Sertoli's cells and inactive germ cells resembling those found in sexually immature rats. These changes are completely and rapidly reversible when adequate food supplies are restored (Mason 1933; Siperstein 1921).

Testicular atrophy of varying severity (maturation arrest, hypocellularity, and "Sertoli-only") has been reported in rats with spontaneous diabetes mellitus (Wright et al. 1982; Murray et al. 1983), alloxan diabetes (Schöflling et al. 1967; Scaffidi and Rotolo 1974), streptozotocin diabetes (Oksanen 1975), diet-induced diabetes (Rosenmann et al. 1974), and diabetes secondary to 95% pancreatectomy (Lema et al. 1965). The mechanism of the degeneration change is not clear. In diabetic men, testicular atrophy has been attributed to diffusion or perfusion problems secondary to basement membrane thickening, diabetic microangiopathy, or atherosclerosis. Because the seminiferous tubules are avascular, nutrients must diffuse through the basement membrane of the tunica propria to nourish the germinal epithelium. Although severely atrophic tubules (Sertoli-only) usually have thickened tunica propria and basement membranes, these changes may be secondary to atrophy rather than causative. Microangiopathy and atherosclerosis have not been clearly demonstrated in diabetic rats and, therefore, are unlikely etiological factors. Several studies with alloxan diabetic rats appear to implicate the pituitary. Testicular atrophy can be prevented or ameliorated by the administration of cortisone, HCG, insulin, or ascorbic acid, but not by testosterone-propionate (Scaffidi et al. 1975; Chatterjee 1966; Chatterjee and Mukherji 1966).

According to Mason (1933), testicular atrophy secondary to E-avitaminosis in the rat may be divided into five morphological stages: (1) chromolysis and fusion of spermatozoa; (2) liquifaction and segregation of the chromatin of spermatids and secondary spermatocytes to one side of the nucleus (i.e., "crescent-like" nuclear changes) (Fig. 269); (3) fusion of crescent-like spermatids and secondary spermatocytes into multinucleated giant cells (Fig. 270); (4) chromolysis of the primary spermatocyte and spermatagonia giant cell nuclei; and (5) "Sertoli-only" change. Because the tubules are not affected simultaneously, different stages may be seen in adjacent tubules. The initial stages of damage occur after 50-100 days of E-avitaminosis; the entire degenerative process requires an additional 35-50 days. According to Mason (1933), the "crescent-like" nuclear changes and more extensive giant cell formation are pathognomonic of E-avitaminosis. The degenerative changes are irreversible even if vitamin E is restored a week or more prior to any histological evidence of injury (Mason 1940).

Testicular atrophy secondary to A-avitaminosis is not as severe as that caused by E-avitaminosis (Mason 1933). The more mature sperm-forming cells are sloughed and most tubules appear in a state of maturation arrest with only the spermatagonia and primary spermatocytes spared (Fig. 269). However, spermatogenesis does not cease entirely; a few tubules continue to attempt an abortive type of sperm maturation (Mason 1933). These degenerative changes are reversible in 1 or 2 months with vitamin A supplementation. Complete atrophy does not occur because rats die from the other sequelae of A-avitaminosis before the residual germ cells are affected. If rats are supplemented with retinoids other than vitamin A, they do not die of these other sequelae and develop irreversible testicular atrophy (Thompson 1970).
Several elemental deficiencies have profound adverse effects on spermatogenesis. Histochemical studies indicate that zinc is incorporated into spermatids and spermatozoa. Acute zinc deficiencies secondary to inadequate dietary zinc, presence of zinc chelators, or increased levels of other related elements cause maturation arrest of the germinal epithelium. Chronic zinc deficiency causes irreversible complete atrophy in postpubertal rats; however, the deficiency is reversible by zinc repletion in prepubertal rats (Mason et al. 1982). Manganese and selenium are also incorporated into spermatozoa. Chronic dietary manganese deficiency causes maturation arrest at the spermatid level (Orent and McCollum 1931), with point of incorporation during spermiogenesis (Gunn and Gould 1970). Although testicular size may decrease to 50% of normal, atrophy is reversible by manganese repletion (Gunn and Gould 1970). Dietary selenium deficiency in the rat causes spermatid midpiece breaks and poor sperm motility, but seminiferous tubule histology is essentially normal (Gunn and Gould 1970).

Ionizing radiation and numerous drugs prevent spermatogenesis and cause testicular degeneration in the rat. These have been reviewed elsewhere (Ellis 1970; Timmermans 1974; Gomes 1970; Jackson 1925) and are outside the scope of this review.

Comparison with Other Species. Degenerative changes within rat testes have been more extensively studied than those in other species, including man. Cryptorchidism is probably the most intensively studied mechanism of testicular atrophy in man. Cryptorchid human testes undergo degenerative changes similar to those in the rat but end-stage damage requires many years. In man, lack of tubular growth and decreased numbers of spermatogonia can be recognized by the 3rd year of life. "Sertoli-only" change does not occur until after puberty. Eventually, thickening of the basement membrane and tunica propria become prominent (Nistal et al. 1980; Wong et al. 1973; Levin 1979). Cryptorchidism in man is associated with an increased incidence of germ cell neoplasms in both the ipsilateral and contralateral testes (Kuber 1982).

Senile testicular atrophy occurs in man. It is characterized by decreased testicular mass, decreased or absent spermatogenesis, peritubular fibrosis, and mild Leydig's cell hyperplasia; Sertoli-only change may also occur. Frequently, normal spermatogenesis is present focally. Testicular atrophy in the diabetic man is characterized by either maturation arrest of hypocellularity. Although Sertoli's cells become relatively more numerous, Sertoli-only change is uncommon. Some degree of tubular basement membrane or tunica propria thickening is usually present. Leydig's cells are generally present in normal numbers. Interstitial tissue may be normal or increased. These changes are often associated with testicular arteriosclerosis (Federlin et al. 1965; Irisawa et al. 1966; Faerman et al. 1972; Singhal et al. 1969; Chokyu 1965; Schöffling 1971; Thiel et al. 1981). Diabetic testicular atrophy has also been described in other species including obese hyperglycemic AO mice and diabetic Chinese hamsters (Schöffling 1971; Schöffling et al. 1967).

Vitamin E, selenium, zinc, and manganese deficiencies are laboratory states that are of theoretical significance; these deficiencies have not yet been conclusively demonstrated in man. Human vitamin A deficiency has not been associated with testicular pathology. The effects of inanition on the human testis include mild atrophy with near-complete absence of spermatozoa, peritubular fibrosis, and increased interstitial cells (Keys et al. 1950; Jackson 1925). Multinucleated giant cells arising from germinial epithelium do not occur in human testicular atrophy regardless of its etiology.

Most nutritional deficiencies affect common laboratory animals in a manner similar to that described for the rat. Vitamin E deficiency is an exception. In the rat, testicular damage secondary to E-avitaminosis is irreversible. On the other hand, degenerative changes occur slowly and are reversible in the hamster. Changes include progressive reduction in the size of seminiferous tubules and in the height of the germinal epithelium; multinucleated giant cells, sloughing of the germinal epithelium, and in situ germ cell necrosis are markedly decreased. Accumulation of acid-fast pigment in Sertoli's cells is markedly increased in the hamster (Mason and Mauer 1975). Similar findings have been reported in the testes of vitamin E deficient guinea pigs and rabbits. The mouse testis is resistant to damage from vitamin E deficiency (Mason and Horwitt 1972).

References

Atrophy, Testis, Rat


Appendix E
Spontaneous Gastric Erosions and Ulcerations in BB Wistar Rats1,2,3

James R Wright Jr, Allan J Yates, Hari M Sharma, and Pierre Thibert

Summary | One hundred thirty-four BB Wistar diabetic rats, 31 nondiabetic siblings, and 30 Wistar rats were necropsied. Gastric erosions and ulcers were observed in 43 (32.1%) of the diabetic rats and three (9.7%) of their nondiabetic siblings. None of the Wistar rats showed evidence of gastric mucosal injury. Lesions were most frequently found in the thick-walled, glandular portion of the stomach and were morphologically consistent with stress ulcers. BB Wistar rats may be valuable for studies on gastric stress ulcers.

Key Words | Diabetes mellitus — Gastric mucosa — Ulcer — Hattun species

The BB Wistar [Bbk(WI)] rat, a promising animal model for human diabetes mellitus, was developed several years ago from an outbred line of Wistar rats at the Bio Breeding Laboratories in Ottawa, Canada (1). Diabetes in the BBW rat occurs spontaneously between 30 and 120 days of age in 30 to 55% of the offspring of diabetic parents. The mechanism of transmission is unknown.

The diabetic syndrome is characterized by hyperglycemia, glycosuria, ketocidosis, insulinopenia, glucagonemia, and hyperlipemia. Without insulin therapy, diabetic rats develop rapid weight loss with polyuria resulting in dehydration. Death frequently follows within 1 to 2 weeks. In addition to these features, the early onset of diabetes in the absence of obesity more closely mimics the clinical syndrome of juvenile-onset diabetes in man than do other animal models. Unfortunately, the BBW rat is susceptible to a wide variety of degenerative, inflammatory, and neoplastic disorders that appear unrelated to diabetes mellitus (2). We report here in detail one of these complications, gastric ulceration.

Materials and Methods

A breeding colony of BB Wistar rats was established at Ohio State University from the parent colony at the Health Protection Branch of the Canadian Government. Animals were shipped by commercial airlines from Ottawa to Columbus, Ohio where they were originally housed in a conventional animal housing facility, and later in a semi-barrier housing facility. The animal room was maintained under slight positive pressure at 22°C and was supplied with fresh air passed through high efficiency particle filters at a rate of 16 air changes per hour. Humidity varied between 45% and 55%, and a constant photoperiod was maintained with a 12-hour light-dark cycle. Rats were housed as mating pairs in 27.9 x 48.3 x 17.8-cm polyurethane cages with hardwood chip bedding.* All rats had access to commercial rat diet† and water ad libitum.

All rats were weighed and monitored daily for ketones* and glucose* in the urine with laboratory test strips. Glycosuria was estimated semiquantitatively on a scale from 0 to 4 plus; ketosuria was recorded as zero, small, or large. Protamine zinc (U-40) insulin* was administered subcutaneously each morning to maintain 4-1- glucose (without ketosis) in the urine. This treatment regimen was used because our colony was being employed in other studies involving the complications of long-term, poorly controlled diabetes. The initial dosage was determined on the basis of body weight and then adjusted according to individual responses to therapy determined by urinalysis. Nondiabetic siblings, BB Wistar rats that did not develop diabetes, and commercially purchased outbred Wistar rats* were housed under identical conditions but did not require insulin therapy.

All rats which either died spontaneously or were killed for experimentation were necropsied. Most major organs were examined for gross abnormalities, weighed, fixed in neutral phosphate buffered formalin, and processed for light microscopy. The stomach was opened anteriorly by means of a longitudinal incision from the cardiac sphincter to the proximal duodenum. Lumenal contents
were examined, and the mucosal surface was washed and carefully inspected with the naked eye. Because a dissecting lens was not employed for this examination, it is possible that very small or completely healed lesions were overlooked. The intestines also were opened and carefully examined. Red-tinted luminal contents were frequently tested for blood with test strips, but this was not routinely done. Tissue from any abnormal areas as well as other representative portions of the stomach, large and small bowels were fixed and processed for light microscopy. When multiple gastric lesions were present, three to five areas representative of these were sampled. Paraffin embedded tissues were sectioned and routinely stained with hematoxylin and eosin. Five serial sections of each tissue sample with a lesion, and one section of samples with no gross lesion were examined histologically. No attempt was made to quantitate the number of lesions in each animal, nor were distinctions made between erosions (those lesions involving the mucosa only) and ulcers (those lesions perforating the muscularis mucosae). Several representative sections of stomach lesions with a light brown pigment were also stained with Gomori stain for iron, oil red 0 for lipofuscin, and periodic acid Schiff (PAS) stain.

Several comparisons were made to determine whether the quality of diabetic control affected the incidence of gastric lesions. The frequencies of gastrointestinal lesions were compared in animals dying spontaneously with those killed for experimentation. Treatment records for individual diabetic rats also were examined. Since stress ulcers are considered an acute process, we utilized only the records for the day prior to death for statistical analysis. The following parameters were compared: (1) insulin dosage received, (2) level of glycosuria, and (3) presence or absence of ketonuria. All frequency comparisons were made using a two-tailed standard normal (Z) test for the difference between two proportions (3). Means were compared by two-tailed Student's t-test.

Results
Necropsies were performed on 134 BBW diabetic rats (107 male and 27 female), 31 non-diabetic siblings (17 male and 14 female), and 30 Wistar rats (all male). Gastric erosions and/or ulcers were observed in 43 (32.1%) of the diabetic rats and three (9.7%) of the non-diabetic siblings, but none were seen in the Wistar rats. Differences in the frequencies of lesions in the two groups of BBW rats were significant (Z = -2.51, p = 0.012), but there was no difference in the frequencies in males (31.8%) and females (33.3%) diabetic BBW rats. Two of the three non-diabetic BBW rats with lesions were female.

No significant age differences were observed between groups of rats. The mean ages of BBW rats with lesions, BBW rats without lesions, and Wistar rats were 265 ± 91 days (range was 108 to 454 days), 243 ± 93 days (range was 91 to 464 days), and 255 ± 200 days (range was 60 to 551 days), respectively. The mean age of onset of diabetes was 92 ± 24 days for rats with gastric lesions and 108 ± 52 days for rats without lesions.

Lesions were either pinpoint in size and shape, or long (up to 10 mm) and narrow (Figure 1). They were multifocal in all but three rats, usually present only in the thick-walled glandular portion of the stomach, and most frequently occurred at the bases of the rugae. However, two rats also had erosions in the thin-walled squamous portion of the stomach. The ulcer base frequently was stained dark brown by the acid digestion products of the exuded erythrocytes. The surrounding mucosa was occasionally hyperemic. The gastrointestinal lumina of severely affected rats usually were virtually devoid of solid food particles but contained variable amounts of blood stained fluid.

The small lesions were restricted to the mucosa and histologically consisted only of coagulation necrosis (Figure 2). Marked hyperemia usually was found peripheral to these foci which frequently contained a light brown pigment that was negative with Gomori stain for iron, oil red 0 for lipofuscin, and PAS. This suggested that the pigment was heamatin, an iron-free breakdown product of hemoglobin (4). Leukocytic margination was found in the submucosal blood vessels in these mild lesions, but in more severely affected foci, a neutrophil-rich exudate was present throughout the necrotic mucosa. In still more severe lesions, the necrotic tissue had sloughed, resulting in either an erosion or an ulcer. Although no distinction between erosions and ulcerations was made for quantitative studies, lesions of both types were observed, and it is our impression that erosions predominated.

A significantly higher incidence (39.3%) of lesions was observed in the 102 rats which died spontaneously when compared to the incidence (9.3%) in the 32 diabetic rats killed for experimentation (Z = 3.51, p < 0.001). The mean dosage of insulin on the day prior to death (Table 1)
Gastric erosions are frequent findings in routine necropsies of laboratory rats (12–14). Therefore, it is not surprising that we found no lesions in the thirty Wistar rats that were housed with the BBW rats. Since similar gastric lesions were observed in both diabetic and non-diabetic BBW rats, it is likely that the BBW strain, regardless of the diabetic condition, has an increased susceptibility to gastric mucosal injury. However, the difference in incidence of the lesions in the BBW siblings indicates that the diabetic state favors the expression of these lesions. Furthermore, gastric lesions were more frequently observed in diabetic rats that died spontaneously than in those that were killed for experimental reasons. This suggests that poorly controlled diabetes may serve as a stressful condition that initiates the necrotic process in the gastric mucosa in these rats.

Since all of the BBW diabetic animals in this study had glycosuria, they were by most standards poorly controlled. Indeed, we adjusted their insulin on a daily basis to maintain them with 4+ glycosuria but without ketosuria. However, some deviations from these levels were unavoidable. In order to determine if gastric lesions were related to the degree of glycosuria, ketosuria, or insulin dosage, the relevant data from the treatment records for each rat’s final day of life were grouped according to whether lesions were present or absent. Both groups of rats had similar levels of glycosuria, and ketosuria was only slightly more frequent in diabetic rats with ulcers (Table 1). However, mean insulin dosage for rats with ulcers was significantly higher than without (1.47 ± 0.82 IU) with 0.93 ± 0.89 IU) gastric lesions (ps < 0.01). Mean levels of glycosuria were essentially the same for both groups. Ketosuria was more frequently observed in rats with ulcers, but this was not statistically significant.

Discussion

Gastric erosions and ulcers can be divided into two categories, acute and chronic. Acute lesions (ulcers and erosions) usually have poorly defined margins and frequently are stained dark brown due to the acidic digestion of erythrocytes. They are commonly multifocal and have no preferred site in the gastric mucosa. Chronic lesions, on the other hand, usually are solitary and are almost exclusively located in the gastric antrum or lesser curvature. They typically have a sharply demarcated border and a clean-appearing base (6). The appearance of the lesions in the BB Wistar rats was typical of acute mucosal injury.

The pathogenesis of such gastric lesions is complex and still has not been completely elucidated. Diabetes is known to affect gastrointestinal tract structure and function (6), but it is unlikely that the diabetic state per se is entirely responsible for the gastric lesions in the BB Wistar rat. Numerous factors affect the resistance of gastric mucosa to injury (7), and although it is theoretically possible that some of these mechanisms may be compromised by the diabetic state, this has not been established. In fact, a decreased incidence of gastrointestinal ulcers in human diabetes has been reported (8) which may be due to a subnormal capacity to secrete gastric acid (9–10).

A recent study may offer some insight into the mechanism of the mucosal injury in the BB Wistar rat. It reported a significant increase in antral and serum gastrin levels in obese genetically diabetic mice when compared to littermate controls (11). The association of gastrin hypersecretion and the occurrence of multifocal gastric ulcers, for example, the Zollinger-Ellison Syndrome in man (5), is well established. Although gastrin levels have not been studied in the BB Wistar rat, in view of our findings this would be very interesting.

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Because of the clinical importance of stress ulcers, there has been considerable effort to develop a reliable experimental model for gastric ulceration. Erosions have been induced in rodents by a variety of experimental techniques including electric shock with and without starvation, pyloric ligation, variable means of physical restraint with and without starvation, and restraint with cold ambient temperature (15). However, all of these have shortcomings as models of the human disease. For example, many of them produce lesions predominately in the non-glandular portion of the stomach and most of the lesions are restricted to the mucosa. It is thus apparent that a spontaneous model more closely mimicking human acute gastric stress ulcers would be quite valuable.

References

Appendix F
Supradiaphragmatic Accessory Lobe of the Liver in BB Wistar Rats

JAMES R. WRIGHT, JR., HARI M. SHARMA, ALLAN J. YATES, BRUCE M. THOMPSON, AND PIERRE THIBERT
Department of Pathology, The Ohio State University, College of Medicine, Columbus, Ohio 43210; J.H., Jr., H.M.S., A.J.Y., and Animal Resources Division, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada K1A 0L2 (U.M.T., P.T.)

ABSTRACT Supradiaphragmatic accessory livers were observed in two closely related rats in a series of 172 necropsies on BB Wistar rats. The gross and histologic appearance of both accessory lobes is described. This abnormality has been reported in only one other inbred strain of rats where it also arose with a very low incidence. As in the previous report, the pattern of occurrence of these accessory lobes suggests a mode of inheritance that is either polygenic or autosomal recessive with low penetrance.

A wide variety of gross developmental abnormalities of the liver has been reported infrequently in man (Levy et al., '79; Cullen, '25), but these appear to be exceptionally rare in the rat (Wilson, '78). The following is a report of two observations of supradiaphragmatic accessory livers in BB Wistar (BBW) rats.

The BB Wistar (BBW) rat, a strain that was developed from an outbred line of Wistar rats at the Bio Breeding Laboratories in Ottawa, Canada, develops a syndrome that closely mimics human juvenile-onset diabetes. The BBW syndrome is characterized by hyperglycemia, glycosuria, ketoadiposis, insulinopenia, and hyperlipemia (Nakhooda et al., '77; Nakhooda et al., '78). Onset of diabetes occurs spontaneously at 60-160 days of age in 30% to 55% of the offspring of diabetic parents. Onset is abrupt with rapid weight loss due to dehydration as a result of polyuria. Death occurs within several weeks if insulin therapy is not initiated.

MATERIALS AND METHODS
A total of 172 BBW rats (139 diabetic and 33 nondiabetic) that died spontaneously or were sacrificed for experimentation were necropsed. Organs were removed en block, dissected, weighed, fixed in neutral phosphate-buffered formalin, and processed for light microscopy. Tissue sections were stained with hematoxylin and eosin.

RESULTS AND DISCUSSION
Two supradiaphragmatic accessory livers were observed in the 172 BBW rats necropsied in our study (i.e., 1.16%). Grossly, the accessory livers in both rats were attached to the rostral right hemidiaphragmatic surface (Fig. 1). In both rats the accessory lobe was approximately 1.5 cm in diameter. The diaphragm appeared grossly normal. Histologically, there was a transition from skeletal muscle fibers to dense regular connective tissue to dense irregular connective tissue near the site of attachment of the accessory lobe. The histological appearance of the accessory lobes differed markedly in the two rats.

The first observation was in a 341-day-old male BBW diabetic rat that died spontaneously. The accessory liver was severely congested, multifocally hemorrhagic and had numerous atypical large blood vessels. Extensive areas of fibrosis partitioned the hepatic parenchyma (Fig. 2). Some fibrous areas showed focal bile duct hyperplasia, nonspecific chronic periportal inflammation, and hemosiderin-filled macrophages (Fig. 3). The capsule of the accessory liver was thickened and merged with the dense connective tissue.
The second observation was in a 415-day-old BBW nondiabetic rat that was sacrificed. Although grossly very similar to the first case, the histological picture was quite different. The accessory liver was surrounded by a thin capsule; fibrosis, bile duct hyperplasia, and congestion were not present; and only a few foci of insignificant periportal chronic inflammation were seen. The parenchyma was essentially normal. Several areas showed limited extramedullary hematopoiesis. This second accessory liver was histologically similar to normal liver tissue.

Complete pedigrees were not available for either of these rats, but we were able to determine that both rats were closely related. Both of the parents of the nondiabetic rat with the accessory lobe were litter mates of the diabetic rat.

A wide variety of developmental abnormalities of the liver have been reported in man (Leevy et al., '79; Cullen, '25). Two types of accessory lobes were described by Grosfeld and Clatworthy ('79a): the Reidel lobe, a tongue-like downward extension of the right lobe of the liver, and accessory lobes projecting into abdominal wall defects (e.g., omphalocele). In addition, ectopic liver tissue in the gall bladder, suspensory ligament, adrenal gland, splenic capsule, umbilicus, or lung have been reported as incidental findings (Leevy et al., '79; Cullen, '25). Hepatic hemartomas are occasionally seen in man, but these are typically located in the right lobe of the liver (Grosfeld and Clatworthy, '79b). All of these differ from the supradiaphragmatic accessory livers present in the BBW rats.

Congenital diaphragmatic herniation can result in an intrathoracic liver surrounded by incompletely developed diaphragm muscle. This pattern was not seen in the BBW rats but has been reported in about 20% of the offspring of irradiated short-eared mice (Snell, '35).

Developmental hepatic abnormalities are extremely rare in the rat (Wilson, '78). Only one previous study (Machado and Luzzio, '72) has reported accessory supradiaphragmatic lobes in that species. These occurred in the right hemithorax in 9 of 2881 (0.31%) Gunn-derived rats. The accessory lobe was independent of both the jaundice and hydronephrosis traits frequently seen in these animal. Since one of our two animals was a nondiabetic sibling, this indicates that this is a strain-related abnormality rather than being linked.
SUPRADIAPHAGMATIC ACCESSORY LIVER IN BBW RATS

Fig. F.2 Photomicrograph of an accessory liver lobe in the first diabetic rat showing extensive areas of fibrosis and abnormal lobular architecture. H & E stain, original magnification, x 25.

Fig. F.3 Fibrous septum showing bile duct hyperplasia in the accessory lobe of the first rat. Hematoxylin-laden macrophages are present near the large blood vessel in the center of the septum. Severe congestion is seen in the dilated hepatic sinusoids. H & E stain, original magnification, x 25.
with diabetes, the trait for which the BBW strain was inbred.

Machado and Lozzo's ('72) description of the microscopic appearance of the accessory lobes in Gunn-derived rats is very similar to the picture in our first rat except that they did not see marked fibrosis or bile duct hyperplasia. They reported poorly defined hepatic plates, congestion, hemorrhage, and limited fibrosis around atypical blood vessels as their predominant findings.

Machado and Lozzo ('72) concluded that this may be an inheritable trait in Gunn rats and if so may involve a mode of inheritance that is either polygenic or autosomal recessive with low penetrance. Our study offers further evidence for a genetic etiology. It is well established that inbreeding raises the frequency of expression of rare genes. Since this trait has only been observed in two studies and both were with highly inbred strains of rats, this is strong circumstantial evidence for a genetic basis of this abnormality.
Appendix G
CENTRAL PONTINE MYELINOLYSIS FOLLOWING SALINE TREATMENT OF A DIABETIC RAT FOR DEHYDRATION

By

J. R. WRIGHT, JR., A. J. YATES, H. M. SHARMA and P. THIBERT

The Ohio State University College of Medicine, Department of Pathology, Divisions of Neuropathology and Anatomical Pathology, Columbus, Ohio 43210, U.S.A.

and

Animal Resources Division, Health Protection Branch, Health and Welfare, Canada, Ottawa, Ontario, Canada K1A 0L2

INTRODUCTION

The BB Wistar (BBW) rat, a promising new model for human juvenile-onset diabetes mellitus, was derived from an outbred line of Wistar rats at the Bio-Breeding laboratories in Ottawa, Canada (Nakhooda, Like, Chappel, Murray and Marliss, 1977). The diabetic syndrome in BBW rats is characterized by hyperglycaemia, glycosuria, ketoacidosis, insulinopenia, glucagonaemia, and hyperlipaemia (Nakhooda et al., 1977; Nakhooda, Like, Chappel, Wei and Marliss, 1978). Spontaneous diabetes occurs in 30 to 55 per cent of the offspring of diabetic matings. The onset is abrupt with rapid weight loss due to dehydration as a result of polyuria. If insulin therapy is not initiated immediately, death occurs within 1 to 2 weeks. Usually, the rats are easily maintained on insulin for 6 months to 1 year after onset of diabetes. However, at this point maintenance often becomes difficult; the rats lose weight rapidly (up to 25 to 40 per cent over a few weeks), become dehydrated and emaciated, and glycosuria disappears (unpublished observation). Ten of these rats were treated with subcutaneous injections of normal saline and their insulin treatment was discontinued. Despite therapy the animals died. One such casualty is the basis of this brief report.

MATERIALS AND METHODS

A male BBW insulin-dependent diabetic rat, 333 days old at the time of death, had been progressively losing weight over several weeks, and became severely dehydrated. The animal received a total of 15 ml of normal saline (0.9 per cent NaCl) as 2 to 3 ml subcutaneous injections per day during the week before its death in an attempt to rehydrate it. The rat died despite therapy (238 days after the onset of diabetes). At necropsy, the rat was thin, emaciated, and weighed only 255 g (normal is approximately 450 g) at death. A drop of blood placed on a Dextrostix (Ames Co., Elkhart, Indiana) reagent strip was negative for sugar. A complete gross post-mortem examination revealed only testicular atrophy, a common finding in older rats of this strain (Wright, Yates, Sharma, Shim, Tigner and Thibert, 1982). The brain appeared grossly normal and was of normal weight (1.67 g). Tissue specimens from all organ systems were fixed in neutral phosphate buffered formalin, and processed for light microscopy. Sections of each organ were stained with haematoxylin and eosin (HE);
sections of brain were also stained with luxol fast blue and Bodian's stain for axons. All organs were histologically normal except the testes (which were atrophic) and the brain. A lesion in the pons involved most of the basal portion and extended into the tegmentum and the base of the middle cerebellar peduncles (Fig. 1). The myelin in this area was almost completely destroyed and macrophages were common (Fig. 2). Necrotic cells with nuclei demonstrating pyknosis and karyorrhexis were scattered throughout the lesion but no oligodendrocytes could be identified with certainty. There were a few cells with large nuclei and a moderate amount of granular eosinophilic cytoplasm which may have represented degenerating neurons; however, neurons were numerous and a few axons traversed even the most damaged area (Fig. 3). There were no reactive astrocytes or evidence of either acute or chronic inflammation and the vessels were normal. One small focus with similar histological features was seen in the corpus callosum.

Nine other rats were treated for dehydration with subcutaneous normal saline, but none of these received more than 9 ml. After a single dose of saline (2 to 8 ml) 4 died within 1 day and 1 within 2 days; 3 lived for 3 to 6 weeks after such treatment. One other received 9 ml normal saline over the last 4 days of life. Brains were examined histologically in all of these rats, but none had evidence of central pontine myelinolysis.

DISCUSSION

Central pontine myelinolysis (CPM), a rare disease of unknown etiology, was first reported by Adams, Victor and Mancall (1959). Since then, over 150
Fig. 6.2 Central pons with macrophages and several unidentifiable necrotic cells showing karyorrhectic and pyknotic nuclei. Luxol fast blue. × 630.

Fig. 6.3 Central pons with axons traversing the area. Bodian's silver stain. × 630.

Human cases have been reported (Wright, Laureno and Victor, 1979). The typical lesion is a single symmetrical focus of grey softening in the centre of the upper and middle pons. Histologically, there is a sharply defined area of myelin breakdown with relative sparing of nerve cells and axons, although some axonal degeneration and nerve cell loss may be present in the very centre
of the lesion. Macrophages containing sudanophilic lipids and reactive astrocytes may be numerous within the lesion, but oligodendrocytes are absent. Neither vascular disease nor inflammation is seen in CPM and therefore cannot be involved in the pathogenesis (Oppenheimer, 1976). The histological findings in this case are similar to those seen in human cases of CPM.

In man, CPM is frequently associated with alcoholism and malnutrition. Recently, electrolyte abnormalities have been implicated in the pathogenesis of CPM (Wright et al., 1979). In fact, many investigators now believe that it is an iatrogenic disorder due to the rapid correction of hyponatraemia (Finlayson, Snider, Olivia and Gault, 1973; Tomlinson, Pierides and Bradley, 1976; Leslie, Robertson and Noreen, 1980; Pogacar, 1980). Some evidence suggests that slower correction of hyponatraemia does not lead to CPM (Leslie et al., 1980). This pattern is consistent with the clinical course (i.e. rapid correction of dehydration) seen in this BBW rat prior to its death.

A recent study by Kleinschmidt-DeMasters and Noreen (1982) has shown that CNS demyelination can be induced by fluctuations in the serum electrolyte concentrations in rats. In that study, rats were made hyponatraemic by the administration of vasopressin and water for 3 days and then treated with hypertonic saline. Rats were killed 5 days later. They reported demyelination with sparing of neurons and axons in regions of the brain with a rich admixture of grey and white matter. Sites of lesions included corpus striatum, claustrum, external capsule, cerebral neocortex, anterior commissure, hippocampus and its fimbria, thalamus, periaqueductal grey, mid-brain reticular formation, red nucleus, and brainstem. In severely affected rats, neuronal loss and necrosis were present in the centre of the lesions. Although the lesions produced in that study are histologically similar to CPM, they are diffuse rather than localized to the pons as in our case.

CPM has been reported in man several months after the onset of clinical diabetes and concurrent with fluid therapy for dehydration (Behar, Bental and Aviram, 1964). We can only speculate as to why CPM might occur in this BBW diabetic rat and yet has not been produced experimentally or observed incidentally in other animals. First, CPM is more common in chronic nutritionally deficient individuals (Oppenheimer, 1976), and poorly controlled diabetes mellitus is such a condition. The emaciation and dehydration that frequently precede the death of BBW diabetic rats may set the stage for the development of CPM. Secondly, the diabetic state causes additional osmotic stress (Aloia and Nilakantan, 1973). We postulate that the combination of these conditions followed by rapid treatment with relatively large doses of saline caused CPM in this case. Nine other diabetic rats which did not develop CPM were treated with normal saline injections but none received as much saline as this animal, and their durations of therapy were all shorter. This suggests that both the dosage schedule of saline and the survival time following initiation of therapy may be critical.

It is probable that many factors were involved in this incidental finding in a diabetic rat, but the significance of this report is that CPM has not, so far, been experimentally induced in animals (Oppenheimer, 1976). This study suggests that this can be done.
Central pontine myelinolysis (CPM) was observed at necropsy in an ema­
ciated 333-day-old BB Wistar insulin-dependent diabetic rat that had been
-treated for dehydration by subcutaneous injection of normal saline. There was
a large area of nearly complete demyelination involving most of the basal
portion of the pons, part of the tegmentum, and the base of the middle cere­
obellar peduncles. There was a relative sparing of neurons and axons, but some
unidentifiable large cells with degenerative changes in the cytoplasm were
present. No oligodendrocytes or reactive astrocytes were seen and there was no
evidence of inflammation or infarction.

Although diffuse demyelination can be induced in rats by rapid fluctuation
of serum electrolytes, no studies have experimentally induced central pontine
myelinolysis nor has it been observed incidentally in animals previously.

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Appendix H
Hematological Characteristics of the BB Wistar Rat

James R. Wright, Jr.; Allan J. Yates; Niranjan T. Shah; John C. Neff; David W. Covey
Ohio State University
College of Medicine
Department of Pathology
Columbus, Ohio 43210

Supported by Samuel J. Hessler Research Scholarship.

Pierre Thibert
Chief, Animal Resources Division
Health Protection Branch
Health and Welfare — Canada
Ottawa, Ontario
Canada K1A 0L2

Summary

Complete blood counts, differential white blood cell and platelet counts were performed on male and female BB Wistar diabetic rats (BBWd), their nondiabetic siblings (BBWnd) and outbred Wistar rats of the line from which the BB Wistar rats were derived. Most of the observed changes were strain-related (those present in both BBWd and BBWnd but not in control rats) rather than diabetes-related (those in BBWd but neither BBWnd nor control rats) and therefore probably due to the inbreeding process. The BBW strain had significantly lower numbers of white cells and platelets, as well as markedly changed differential white cell counts. Differential counts showed a pattern of lymphopenia, neutrophilia, monocytes and eosinophilia. It is possible that these white blood cell changes contribute to the increased susceptibility to infection reported for the BBW strain. No significant difference in serum immunoglobulin concentrations was found in any of these three groups of rats. Therefore, hypogammaglobulinemia cannot account for the increased susceptibility to infections, but it is not possible to rule out an abnormality in the distribution of immunoglobulin fractions as an etiological factor.

KEY WORDS: BB Wistar rat, spontaneous diabetes, complete blood counts, leukopenia, eosinophilia, platelets, immunoglobulin

Introduction

The BB Wistar (BBW) rat, a strain that was developed from an outbred line of Wistar rats at the

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Hemological Characteristics of the BB Wistar Rat

Bio Breeding Laboratories in Ottawa, Canada, develops a syndrome that closely mimics human insulin-dependent (juvenile-onset) diabetes. The BBW syndrome is characterized by hyperglycemia, glycosuria, ketoacidosis, insulinopenia, glucagonemia and hyperlipemia. Onset of diabetes occurs spontaneously between 60 and 160 days of age in 30 to 55% of the offspring of diabetic parents. Onset is abrupt with rapid weight loss due to dehydration as a result of polyuria. Death occurs within several weeks if insulin therapy is not initiated.

The present study was designed to determine several hematological parameters of the BBW rat and was prompted by several observations made during a previous study. First, the BBW rat, both diabetic (d) and nondiabetic (nd), is very susceptible to a variety of infections. Thus, they may have an abnormality of the immune system which could be reflected in the numbers of circulating leukocytes or concentrations of serum immunoglobulins. Second, the frequent presence of eosinophilic infiltrates and/or mast cells in tissue sections of both BBWd and BBWnd rats may be associated with abnormal numbers of these cells in blood. Third, the high incidence of lymphomas in this model suggested that leukemia might also occur. Finally, histological changes suggestive of autoimmunity in some rats prompted us to examine the protein electrophoretic pattern of the serum for a change in the concentrations of gammaglobulins.

In addition to these specific questions, another goal of this study was to determine whether any hematological parameters of the BBW strain differ from those of the original Wistar stock from which they were derived, and whether these changes (if present) are a result of the inbreeding process or of the diabetic condition.

Materials and Methods

A breeding colony of BBW rats was established by the Pathology Department of the Ohio State University College of Medicine in June of 1979. Rats were transported to Columbus by commercial airlines from the parent colony at Health and Welfare of Canada in Ottawa. Rats were originally housed in a conventional animal facility but were soon moved to a semi-barrier housing facility following an outbreak of bronchopneumonia. At the time of this study, the pulmonary infections were under control, but lowgrade pinworm infections were rarely present. All rats were housed together and were therefore equally exposed to any infective organisms that may have been present. Our barrier facility and animal husbandry techniques have been previously described in detail.

Rats used in hematological studies were anesthetized with ether, and blood (300 μl) was collected in capillary whole blood collectors (Microtainers R:Becton-Dickinson, Rutherford, New Jersey) by inserting a heparinized capillary tube (American Hospital Supply Corp., Miami, Florida) into the periorbital plexus of vessels at the inner canthus of the eye. This technique was chosen because it offers more consistent results than tail vein bleeding. Complete Blood Counts (CBC), differential white cell counts and platelet counts were performed on BBWd (16 male and 11 female), BBWnd (12 male and 10 female) and control (9 male and 10 female) rats. (Control rats were a Wistar line obtained from Bio Breeding Laboratories in Ottawa, Canada.) CBC's and platelet counts were accomplished with a Coulter S-plus. Platelet counts in excess of 700,000 were diluted 1:2 until there was agreement in two of three simultaneous measurements.

Blood for serum studies was also collected by retro-orbital bleeding. Samples were allowed to clot and then centrifuged in a tabletop centrifuge (Model HN:International Equipment Co.) with a fixed-angle rotor at 1500 rpm. The supernatant was pipetted into polypropylene micro sample tubes (Kew Scientific, Columbus, Ohio) and frozen at −40°C until use. Protein electrophoresis was performed on agarose plates at pH 8.6. Total protein was measured by the biuret method. Samples from five rats in each of the six categories were compared by two-way analysis of variance.

Results

Table 1 shows the average body weight, CBC and platelet values for both male and female rats in the three groups studied (BBWd, BBWnd, control). Average rat weights for the three groups did not differ significantly within the same sex but were significantly different across sexes. Although exact birth dates were not known for some of the rats, all were adults of similar ages. Inter-group variation for each variable was examined by two-way analysis of variance (ANOVA) using the unweighed means solution for unequal cell sizes, and paired comparisons were performed by the Waller-Duncan Bayes exact test. The re-
**Table H.1**

Complete Blood Count and Platelet Values*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>n</th>
<th>Body Weight (gm)</th>
<th>WBC (x10^3)</th>
<th>RBC (x10^6)</th>
<th>Hgb (in g/dl)</th>
<th>HCT (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>RDW</th>
<th>PLT (x10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBWd</td>
<td>M</td>
<td>16</td>
<td>380.08</td>
<td>5.86*</td>
<td>8.22*</td>
<td>16.30</td>
<td>41.96</td>
<td>52.29*</td>
<td>19.91</td>
<td>38.11</td>
<td>10.72*</td>
<td>767.69*</td>
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<td></td>
<td>F</td>
<td>11</td>
<td>276.00</td>
<td>2.31</td>
<td>1.06</td>
<td>0.80</td>
<td>4.25</td>
<td>1.81</td>
<td>1.15</td>
<td>1.90</td>
<td>1.65</td>
<td>149.69</td>
</tr>
<tr>
<td>BBWnd</td>
<td>M</td>
<td>12</td>
<td>443.54</td>
<td>7.80*</td>
<td>8.09</td>
<td>16.00</td>
<td>42.46</td>
<td>55.54</td>
<td>21.28</td>
<td>38.30</td>
<td>9.33*</td>
<td>832.23*</td>
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<td>F</td>
<td>10</td>
<td>292.00</td>
<td>6.56*</td>
<td>7.75</td>
<td>16.10</td>
<td>42.15*</td>
<td>52.07*</td>
<td>19.86</td>
<td>38.17</td>
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<td>902.21*</td>
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<td>9</td>
<td>415.33</td>
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<td>16.68</td>
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<td>10</td>
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<td>9.18</td>
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<td>15.38</td>
<td>39.47</td>
<td>53.96</td>
<td>21.04</td>
<td>38.98</td>
<td>5.70</td>
<td>1189.60</td>
</tr>
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</table>

Abbreviations: n = number of rats; WBC = white blood cell count; RBC = red blood cell count; Hgb = hemoglobin concentration; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; PLT = platelet.

The second value in each pair represents the standard deviation.

* Value for BBW rat is significantly different (p < 0.01) from the control value.

** Value for BBW rat is significantly different (p < 0.01) from the BBWnd rat value.

Results of these tests have been summarized in Table 1. All of the differential white cell count variables were compared as above except for the monocyte and eosinophil counts, which were examined using the Z test (Table 2). This test was chosen because the intra-group number of monocytes or eosinophils per rat had a bimodal distribution rather than a normal distribution. Therefore, rat blood smears were considered to show monocytosis or eosinophilia when they exceeded the control rat sex-matched mean monocyte or eosinophil counts by four standard errors of the mean. The ratio of the number of rats with eosinophilia or monocytosis within each group (BBWd and BBWnd) was then compared to the same ratio for the sex-matched control group by the Z test (Table 2).

A general observation is that there are many more strain-related differences (those present in both BBWd and BBWnd but not in control) than diabetes-related (those in BBWd but in neither BBWnd nor control) differences (Tables 1 & 2). More specifically, the BBW strain had significantly lower numbers of white cells and platelets, as well as markedly changed differential white cell counts. Differential counts revealed a pattern of marked lymphopenia, slight neutrophilia, monocytosis and eosinophilia. Some statistically significant changes in red cell indices were also seen, but none were outside of the normal range of other rat strains. Red Cell Distribution Width (RDW) values were significantly higher in the BBW strain (particularly BBWd), indicating a tendency toward anisocytosis.

Gamma globulin concentrations were also compared. The mean concentrations (±S.E.M.) in mg/dl for male rats in the three groups (BBWd, BBWnd and control) were 0.624 ±0.055, 0.944±0.069, and 0.687±0.037 respectively, while in female rats these values were 0.938 ±0.088, 0.948±0.186, and 0.866±0.115 respectively. There were no statistically significant group or sex differences.

**Discussion**

It is difficult to establish general baseline hematological data for experimental rats because of the large number of strains, stocks and sub-
Hematological Characteristics of the BB Wistar Rat

Table R.2

Differential White Cell Counts**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>n</th>
<th>Total WBC</th>
<th>PMN</th>
<th>Lymph</th>
<th>Mono*</th>
<th>Eos*</th>
<th>Baso</th>
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<tbody>
<tr>
<td>BBWd</td>
<td>M</td>
<td>16</td>
<td>5,860**</td>
<td>2,280*</td>
<td>2,760**</td>
<td>108</td>
<td>526</td>
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<td></td>
<td></td>
<td></td>
<td>2,320</td>
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<td>129</td>
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<td>11</td>
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<td>3,000**</td>
<td>177</td>
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<td></td>
<td></td>
<td></td>
<td>3,720</td>
<td>1,320</td>
<td>1,030</td>
<td>147</td>
<td>2,140</td>
<td>14.2</td>
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<tr>
<td>BBWnd</td>
<td>M</td>
<td>12</td>
<td>7,800**</td>
<td>3,000*</td>
<td>3,260**</td>
<td>481*</td>
<td>1,050**</td>
<td>3.2</td>
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<td></td>
<td></td>
<td></td>
<td>2,400</td>
<td>1,260</td>
<td>935</td>
<td>507</td>
<td>942</td>
<td>11.1</td>
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<td></td>
<td>F</td>
<td>10</td>
<td>5,660**</td>
<td>2,110*</td>
<td>2,610**</td>
<td>151*</td>
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<td>3,990</td>
<td>1,750</td>
<td>934</td>
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<td>9</td>
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<td>1,620</td>
<td>10,800</td>
<td>29.0</td>
<td>126</td>
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<td></td>
<td></td>
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<td>2,330</td>
<td>885</td>
<td>2,220</td>
<td>86.7</td>
<td>111</td>
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<td>9,180</td>
<td>1,560</td>
<td>7,410</td>
<td>58.9</td>
<td>149</td>
<td>0</td>
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<td></td>
<td></td>
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<td>3,100</td>
<td>630</td>
<td>2,580</td>
<td>58.6</td>
<td>143</td>
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</table>

**Abbreviations: n = number of rats; PMN = neutrophils/ml; Lymph = lymphocytes/ml; Mono = monocytes/ml; Eos = eosinophils/ml; Baso = basophils/ml.

* The second value in each pair represents the standard deviation.

Statistical analyses were performed to test for differences between BBW and sex-matched control rats. Single asterisk (*) indicates the p < 0.05. Double asterisk (**) indicates p < 0.01.

Rat blood smears were considered to show monocyto sis when they exceeded the sex-matched control mean monocyte count by 4 standard errors of the mean (i.e., counts > 144.6 for males and counts > 123.0 for females). Ratio is the number of rats with monocytes by this criterion. Statistical analysis by 2 test.

Rat blood smears were considered to show eosinophilia when they exceeded the sex-matched control rat mean eosinophil count by 4 standard errors of the mean (i.e., counts > 273.7 for males and counts > 330.2 for females). Ratio is the number of rats with eosinophilia by this criterion. Statistical analysis was by 2 test.

stocks recognized within the species. For this reason, we chose non-diabetic Wistar rats from Bio Breeding Laboratory (from which the BBWd was derived) as our control animal for strain comparison. The comparison of BBWd with BBWnd allows us to determine diabetes-related hematological changes while the comparison of BBWd and nd) with control rats allows us to determine strain-related changes due to the inbreeding process. Tables 1 and 2 show that strain-related changes are more frequent than diabetes-related changes. This is consistent with present knowledge since inbreeding of rats is known to change hematological parameters and since diabetes does not significantly alter major red and white cell indices in man.

The changes in red cell indices indicate either a sex-related or random pattern and none of these values are very far out of ranges considered normal for rat hematological studies. The degree of anisocytosis is greatest in the BBWd, lower in BBWnd, and least in control rats. This is what is to be expected since the degree of anisocytosis is increased in several disease states.

Most of the white blood cell indices are changed in a strain-related pattern, but we saw no evidence of leukemia. White cell indices are of great interest for two reasons: (1) the BBW strain (both diabetic and non-diabetic) has an increased susceptibility to infection and (2) autoimmunity has been implicated in the onset of the diabetic syndrome. Both BBWd and BBWnd have significantly lower white cell counts than control rats (Table 1). This could partially explain the higher incidence of infections and possibly the apparent shortened life expectancy of the BBW rat (unpub-
lished observation). Reich and Dunning have reported a positive correlation between the mean life-span of inbred rat strains and their mean WBC count. 13

The differential white blood cell pattern of BBW shows marked alterations from the control rat pattern (Table 2). The typical pattern in the BBW rat shows increased numbers of PMNs, monocytes and eosinophils as well as a large decrease in the number of lymphocytes. Basophils are rare in rats as they were in all rats in this study.

Most of these differential patterns were reasonably consistent (i.e., approximated a normal distribution), but eosinophilia was either present or absent (i.e., bimodal distribution). This, at first, suggested that parasitic infections were responsible. Several lines of reasoning indicate that this is not the case. Since all three groups of rats were housed together, one would expect a parasitic infection to affect all three groups equally rather than the strain-related pattern seen in this study. Secondly, mild pinworm infection (Syphacia obve-
late) was the only parasitism observed in our colony, even following complete necropsy. Pinworm infections are ubiquitous in most rat colonies but eosinophilia has not been reported in association with such infections. Furthermore, our colony (BBWd, BBWnd and control rats) was occasionally treated for pinworms with Piperazine (W.A. Butler Co.) in the drinking water. Thirdly, eosinophilic infiltrates are occasionally seen in rats with large islets of BBW rats. Finally, the eosinophilic infiltrates are occasionally present in tissue sections. This pattern is more suggestive of strain-related hypersensitivity to some unknown allergen or auto-
antigen.

The other major strain-specific observation in this study was that BBW rats have decreased platelet concentrations relative to Wistar rats (Table 1). This was significant at the p < 0.01 level in both BBWd and BBWnd rats. Although this is a significant change, none of the values are markedly different from values reported in hematological studies of other rat strains.

The mean gamma globulin concentrations in our study are well within the normal ranges reported in other studies. Since we observed no group differences in the concentrations of gamma globulins, hypogammaglobulinemia cannot account for the increased susceptibility to infection in the BBW strain. However, it is not possible to rule out some abnormality in the distribution of immunoglobulin fractions as an etiological factor.

ACKNOWLEDGEMENTS

The authors wish to thank Roachel Tigner for her statistical expertise and Dr. D.A. Senhauer and S. Patel for their advice and assistance.

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Appendix I
DNA EXCISION REPAIR IN SPONTANEOUSLY DIABETIC BB WISTAR RATS

JAMES R. WRIGHT, Jr.*, RALPH E. STEPHENS*, KENNETH C. FORD*, PIERRE THIBERTb and ALLAN J. YATES*

*Department of Pathology, The Ohio State University College of Medicine, Columbus, OH 43210 (U.S.A.) and bAnimal Resources Division, Sir Frederick G. Banting Research Center, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario K1A OL2 (Canada)

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SUMMARY

BB Wistar rats develop a syndrome characterized by spontaneous diabetes mellitus as well as a wide variety of autoimmune, neoplastic, and degenerative disorders which do not occur in the outbred Wistar strain from which they were derived. This syndrome also includes elements of premature ageing (i.e., a markedly shortened lifespan and premature occurrence of diseases associated with ageing). Excision DNA repair capacity which has been reported to be directly proportional to maximal achievable life span was estimated in neonatal BB Wistar and outbred Wistar rats. Excision repair was assayed autoradiographically by determining unscheduled DNA synthesis following UV radiation of passage 3 cultured skin fibroblasts. No difference in excision repair capacity could be demonstrated between the two strains.

Key words: Ageing; BB rat; Diabetes mellitus; DNA repair; Fibroblast

INTRODUCTION

The BB Wistar (BB) rat develops a syndrome that closely mimics human juvenile-onset diabetes [1]. In addition to the spontaneous onset of hyperglycemia and ketonuria, young BB rats also develop a wide variety of developmental, autoimmune, neoplastic, and degenerative disorders which do not occur in the outbred Wistar (W) rat...
strain from which the BB strain was originally derived. Many of these occur in a strain-related pattern (i.e., both diabetic and non-diabetic BB Wistar rats) but are exacerbated by the expression of the diabetic trait. This suggests that the BB strain is not simply a Wistar strain that develops diabetes mellitus. Rather, diabetes appears to be only a part of a larger “BB Wistar syndrome” [2], a syndrome which in many ways also demonstrates elements of premature ageing (i.e., a markedly shortened lifespan and premature occurrence of diseases of ageing). Although the onset of hyperglycemia is heralded by an autoimmune insulitis [3,4], the underlying cause of the “BB Wistar syndrome” is not known.

Various human syndromes characterized by premature ageing and symptoms of diabetes mellitus have been attributed to faulty DNA repair [5–10]. In this study, we tested whether an excision DNA repair defect could account for the “BB Wistar syndrome”, but the results also offer insight into a mechanism of ageing. We chose to investigate UV induced excision (long-patch) repair which requires endonuclease, exonuclease, polymerase, and ligase activities, because the results reflect on multiple DNA repair processes rather than just one type of DNA repair. Differing maximal achievable lifespans in various species have been related to differing excision DNA repair capacities [11]. Because the Wistar rat strain and the BB Wistar rat strain are presumably identical except for the expression of the “BB Wistar syndrome” in the latter, it is in many ways an excellent model to examine this relationship.

MATERIALS AND METHODS

BB Wistar (BB) rats and outbred Wistar (W) rats from which the BB Wistar strain was originally derived were obtained from Health and Welfare of Canada. Breeding colonies of both strains were established at The Ohio State University. Our animal facilities and maintenance procedures have been described extensively elsewhere [12].

Primary fibroblast cell lines were established from each of two neonate offspring of single BB or W rat mating pairs. These cell lines were called BB1, BB2, W1, and W3, respectively. Because BB rats do not develop diabetes until 30–60 days of age, the BB pups were not yet diabetic. Neonatal rats were utilized in this study to maximize repair levels since evidence exists that DNA repair efficiency in rats decreases with ageing [13]. The neonates were sacrificed and their abdomens cleaned with 70% ethanol prior to excision of 1 cm² skin samples. Samples were washed three times in phosphate buffered saline (PBS), placed in 0.5 ml of 0.01% of trypsin, and then minced between two razor blades on a ground glass cutting block for 10 min. Minced dermis was placed in 25-ml digestion flasks containing 5 ml of 0.01% trypsin and then incubated for 30 min in a 37°C water bath/shaker. The contents of the flasks were poured into 50-ml plastic centrifuge tubes and the trypsin was inactivated by the addition of 40 ml of B medium (Eagles MEM supplemented with 1.5X essential amino acids, 2X non-essential amino acids and 1.5X vitamins), containing 10% fetal bovine serum and 0.02% gentamycin (B10 × 2).
Tubes were centrifuged at 1100 rev./min for 15 min and the medium decanted. Pellets were resuspended in 8 ml of B_2_ medium, divided into four T_2_ flasks (Corning Glass Works, Corning, N.Y.), and incubated at 37°C with 5% carbon dioxide. After 12 days, the cells had reached near confluency and were harvested with 0.01% trypsin, split 1:2, and plated (i.e., passage 1). The cells were split 1:2 for each successive passage. Passage 3 cells were used for the experiment because they appeared relatively free of epithelial cell contamination and because passage 3 rodent cell lines have not yet undergone a senescent decline in DNA repair capacity [14].

Passage 3 cells for each cell line were suspended at a concentration of 9 \times 10^4 cell/ml into six 100-mm diameter petri dishes containing ten 22 mm \times 11 mm, coverslips with 0.5 ml cell suspension/cover slip. Cells were permitted to attach and grow for 2 days. The medium was then aspirated and replaced with B medium containing 2 \times 10^{-3} M hydroxyurea. After 15 h, the medium was aspirated and the cells washed twice with PBS. For each of the cell lines, the petri dishes were divided into 3 groups: (a) 2 control plates receiving no radiation, (b) 2 plates exposed to 20 J/m^2 ultraviolet (UV) radiation, and (c) 2 plates exposed to 40 J/m^2 UV radiation. Irradiated and unirradiated plates were then exposed to medium containing 2 \times 10^{-3} M hydroxyurea and 2 \muCi/ml tritiated thymidine (\textsuperscript{3}HdThd, spec. act. = 20 Ci/mmol, New England Nuclear) for 0, 2, 4, 6, and 8 h. After their respective incubations, coverslips were removed from the dishes, washed three times in PBS, fixed for 5 min in freshly prepared Carnoy's solution three times, dehydrated through an ethanol series, mounted on labeled glass slides cell-surface up, and placed overnight in a drying oven. The glass slides were then dipped in a photographic emulsion (0.67% glycerol + 32.67% distilled water + 66.67% Kodak NTB-2) and stored in opaque slide boxes containing calcium carbonate at 4°C. After 6 days, the emulsion coated coverslips were developed with Kodak D_19, washed, fixed, and lightly stained with toluidine blue. Cells were magnified 630X and nuclear grains were counted electronically with a Docuval camera and an Artex 880 counter. The frequency of nuclear grains was determined for approximately 50 non-S phase cells from each coverslip. Corrections for background on each coverslip were made by subtracting the mean number of grains present in adjacent equivalent areas without cells. All analyses were performed on these adjusted grain counts. Three coverslips per cell line were examined for most group and dose interactions. However, only two coverslips were available for several group-dose interactions. The magnitude of scheduled DNA synthesis for each group and dose was equivalent to the average number of grains per nucleus for irradiated cells minus the values for unirradiated cells.

RESULTS

Grains were counted in 11 452 nuclei. In 1767 of these, the grains were too numerous to count reliably, so those nuclei were not included in the analysis. The sample size was thus 9685. The 1767 nuclei dropped from the analysis were predominantly from the higher dosage/longer incubation period conditions. Therefore, the mean grain counts...
Table 1.1

ANALYSIS OF VARIANCE PARAMETERS

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
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<th>F</th>
</tr>
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<tr>
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<tr>
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<td>Error</td>
<td>1794676.9</td>
<td>9625</td>
<td>186.5</td>
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Three-way Analysis of Variance with grain count as the dependent variable and animal, dosage of UV radiation, and time interval as the independent variables; SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-score. Effect of heterogeneity of variances was judged to be minimal because the cell sizes (i.e. n for each treatment) were large and uncorrelated with variances (r = 0.09).

in those conditions are underestimated. A three-way ANOVA was performed on grain counts (Table 1). Figure 1 shows that the magnitude of unscheduled DNA synthesis for both BB Wistar and Wistar rats increased from 0 to 20 J/m², but did not increase at the higher dose. Post-hoc comparisons using the Tukey method confirmed that tritiated thymidine uptake at 40 J/m² was not significantly greater than at 20 J/m² (P >

Fig. 1.1 Unscheduled DNA synthesis (average grains/nucleus) in BB and W rats as a function of fluence at 0, 2, 4, 6, and 8 h of incubation. Repair at 40 J/m² does not exceed that at 20 J/m².
Figure 1.2 Unscheduled DNA synthesis (average grains/nucleus) in BB and W rats as a function of time at a dosage of 20 J/m². Pooled standard errors of the means at 0, 2, 4, 6, and 8 h are 0.28, 0.67, 0.65, 0.63 and 0.72 grains/nucleus, respectively.

0.05, one-tailed). Therefore, the responses of the BB Wistar and Wistar rats for the different incubation periods were graphed using the data for 20 J/m².

Figure 2 shows the average amount of unscheduled DNA synthesis as a function of time in BB Wistar and Wistar rats. The average number of grains increased with incubation time in an approximately linear fashion with saturation beginning to occur at longer time periods. At every time interval, unscheduled DNA synthesis was slightly greater in the BB Wistar rats than in the Wistar rats. To determine whether the two control animals had significantly less uptake of tritiated thymidine (i.e., unscheduled DNA synthesis) than the two BBW diabetic animal, post-hoc comparisons were performed. The Tukey method was used to test the hypothesis that the grain counts for the two control rats were significantly less than the grain counts for the two diabetic rats at each of the 15 dosage X incubation time levels. None of these tests showed significant differences at the 0.05 level (one-tailed).

DISCUSSION

Our experience suggests that a shortened lifespan is part of the “BB Wistar syndrome”. The average lifespan of the outbred Wistar rat is about 26 months, and the maximum achievable lifespan approximately 46 months [15]. Although similar vital statistics are not available for the BB strain, these animals rarely survive longer than 14 months
regardless of the expression of the diabetic trait. Interestingly, many of the hematopoietic and degenerative disorders characteristic of the BB Wistar strain also occur in outbred rats, but not unless the rats are "aged" (2–3 years old). The presence of a wide variety of spontaneous senile lesions occurring in "young" BB Wistar rats is consistent with premature ageing. For instance, BB Wistar rats commonly develop lymphomas [2,16]. In our autopsy series, the mean age of BB rats with lymphomas was 280 days with the youngest being only 6.5 months old. Primary lymph node neoplasms occur sporadically in 2–3-year-old rats [17–19], but have never been reported in a rat younger than 17 months of age [17]. The youngest rat with a lymphoma was a 20-month-old Sprague–Dawley rat [20]. The presence of a markedly shortened lifespan and the premature occurrence of diseases of ageing are strong evidence of premature ageing in BB Wistar rats.

It has been speculated that human diabetes may represent a form of premature ageing [21–23] and many human syndromes of premature ageing manifest symptoms of diabetes mellitus [6]. It is well known that human diabetics also have decreased lifespans and experience some of the diseases of ageing prematurely. Although some recent clinical studies suggest that precise control of blood glucose will delay or prevent diabetic sequelae, there is a large body of evidence suggesting that there is a major genetic component.

Numerous studies have shown that fibroblasts from human diabetics or prediabetics (individuals with strong familial tendency toward diabetes but have not themselves expressed hyperglycemia) are markedly different from fibroblasts from normal subjects. Non-diabetic fibroblasts grown in cultures exhibit greater plating efficiencies [24–26], numbers of passages or cell population doublings before senescence [24,26–29], DNA synthetic abilities [25–26], and log-phase doubling rates [26,30]. Because rat fibroblasts transform rather than senesce [31] these types of studies would be a less meaningful way to evaluate ageing in diabetic rats than in diabetic humans. However, excision DNA repair studies with neonatal rat fibroblasts permit evaluation of maximum achievable lifespan and ageing in BB Wistar rats.

Several studies have demonstrated that a mammalian species capacity for excision DNA repair bears a direct linear relationship to its maximal achievable lifespan [11,32]. This has also been demonstrated in both inbred and outbred rodents of differing longevities. Hart et al. [33], compared excision repair of UV damage in early-passage cultured fibroblasts from two species of outbred mice with differing maximum achievable lifespans, Mus musculus (1250 days) and Peromyscus leucopus (3000 days). Both maximum achievable lifespan and capacity for excision DNA repair were greater in Peromyscus by a factor of 2.5 [33]. Similar results were obtained using fibroblasts from fetal inbred NZB, C3Hf, and CBA/H mice [34] and PHA stimulated splenic lymphocytes from adult inbred NZB and CBA mice [35].

Our findings demonstrate that no abnormality of excision DNA repair exists in BB Wistar rats. This suggests that the differences in longevities are not due to abnormalities of this multiple enzyme requiring system. Obviously, this does not rule out the possibility of this disorder being due to abnormalities in DNA through other mechanisms, or that
there could be isolated repair defects involving certain essential genes. At this point the specific molecular genetic basis for the BB Wistar syndrome remains obscure.

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Appendix J
Pathological Lesions in the Spontaneously Diabetic BB Wistar Rat: A Comprehensive Autopsy Study

James R. Wright, Jr., Allan J. Yates, Hari M. Sharma, and Pierre Thibert

A total of 145 BB Wistar diabetic rats, 46 of their nondiabetic siblings, and 43 outbred Wistar rats were autopsied and the frequency of lesions in all organ systems were determined. Common strain-related lesions included pulmonary infections, granulomas, lymphoid hyperplasia, lymphomas, lymphocytopenia, eosinophilia, supradiaphragmatic accessory lobes of the liver, and prostatic atrophy. These suggest some basic strain-related abnormalities of the immune system that were selected by the process of inbreeding. Diabetes-related lesions were insulin, testicular atrophy, cataracts, hepatic fatty change, pancreatitis, lymphocytic thyroiditis, hyperglycemic brain damage, central pontine myelinolysis, stomach erosions, and idiopathic megacolon. Many of these are sequelae of human juvenile-onset diabetes and support the validity of the BB Wistar rat as an animal model for human diabetes mellitus. The absence of several important sequelae of the human disease (i.e., diabetic nephropathy, athrosclerosis, and severe microangiopathy) suggests a degree of infidelity as a model for human diabetes mellitus. Nonspecific lesions occurring in all three groups of rats included myocardial degeneration and fibrosis, splenic extramedullary hematopoeisis, and chronic progressive glomerulonephropathy.

Our approach to examining the validity of the BB Wistar rat as an animal model for human juvenile-onset diabetes has been to perform detailed autopsies on 145 BB Wistar diabetic, 46 of their nondiabetic siblings, and 43 outbred (non-BB) Wistar rats that died spontaneously or were sacrificed for experimentation. The types and frequencies of all lesions were tabulated by age and group. Some of the results of this study have been published in detail elsewhere. Here we present an overview of our findings. The significance of these is best demonstrated by utilizing the following classification scheme:

1. Strain-related lesions are abnormalities found in diabetic and nondiabetic BB but not in Wistar rats.
2. Diabetes-related lesions are abnormalities found in BB diabetic rats but not in nondiabetic or Wistar rats. These lesions were subdivided into three groups:
   a. Lesions strongly associated with human diabetes.
   b. Lesions weakly associated with human diabetes.
   c. Lesions not specifically associated with human diabetes.
3. Nonspecific lesions are those that occurred in nondiabetic and diabetic BB and Wistar rats with similar frequencies.
4. Organ systems virtually free of significant pathology.

Strain-related lesions

Table I shows the frequency of strain-related lesions in BB rats. These include lymphoproliferative, inflammatory, degenerative, and developmental disorders. Of these, bronchioleumonia is of practical importance because of its relatively high incidence and mortality. Older studies suggest that human diabetics are more susceptible than are nondiabetics to pulmonary infections, but in the BB rat these are strain-, not diabetes-related. The absence of pneumonia in Wistar rats housed in the same facility indicates that the BB strain has an increased susceptibility to pulmonary infections. The incidence diminished considerably when the colony was housed in semibarrier facilities. Mycoplasma appears to be the usual causative agent, but other organisms were occasionally present.

Granulomatous lesions were moderately common in BB rats, but no organisms were seen histologically. Organs frequently involved were lymph nodes, kidney, and pancreas; those infrequently involved were lungs and testes. Although granulomas were usually small, some large lesions occurred in lymph nodes.

Strain-specific lymphadenopathies were common in BB rats in contrast to the uniformly normal lymph nodes in the Wistar rats. Mesenteric lymph nodes in Wistar rats were small and distinct, while in BB rats they were usually fused into one or more longitudinal nodes, some of which were very large. Although there was no uniform histological appearance in these enlarged nodes, most had sinusoidal hyperplasia. Three cell populations predominated in hyperplastic nodes. In some, sinusoids were massively dilated with plasma cells and differed from plasmacytomas only by the presence of occasional cortical follicles. Others

From the Ohio State University College of Medicine, Department of Pathology, Columbus, Ohio; and Animal Resources Division, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada.

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Address reprint requests to Allan J. Yates, M.D., Ph.D., Neuroradiology Unit, Department of Pathology, The Ohio State University, 103 Upham Hall, 473 W 12th Ave, Columbus, OH 43210. 0026-0410/83/3206-0020/$01.00/0

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showed sinus histiocytosis. A third group had mixed cell
types in diluted sinusoids. Most were lympho-depleted,
and germinal centers were rarely seen in the cortical
follicles. Lymph nodes from the Wistar rats in our
study had distinct follicles, frequently with germinal
centers, and never showed the sinusoidal hyperplasia
or lympho-depletion typical of BB rats.

Lymphomas were the most common neoplastic
lesions. They were found in approximately 4% of older
BB diabetic and BB nondiabetic rats but were not seen
in Wistar rats. The mesenteric lymph nodes and the
colon were the most frequent sites of involvement.
Other sites included the mediastinal lymph nodes,
thymus, ileum, liver, pancreas, spleen, lungs, and ova-
ry. Lymphomatous nodes were firm, lobulated, white,
and usually hemorrhagic. Histologically, they had a
diffuse pattern and the cells were histiocytic with
plasmacytoid differentiation. Some were so large that
they caused complete bowel obstruction. Kallman and
Seemayer have previously reported a 12.6% and 1.2%
incidence of mesenteric lymphomas in diabetic and
nondiabetic BB rats, respectively. These tumors histo-
logically were either plasmacytoid or had features of
immunoblastic sarcomas.12

Strain-specific hematological differences were also
found. A comparison with Wistar rats, BB had signifi-
cantly fewer white cells and platelets, as well as
different differential white-cell counts. There was
marked lymphocytopenia, slight neutrophilia, monocyt-
o sis, and eosinophilia. None studied had leukemia.
Recently, it has been shown that the lymphocytopenia
is due to a T cell deficiency.13 These findings are
consistent with the lympho-depleted appearance of the
lymph nodes and the frequent presence of an eosino-
phile infiltrate in inflammatory lesions. No significant
changes in red-cell indices or gamma-globulin concen-
trations were found. The presence of lymphoid hyper-
plasia, spontaneous granulomas in the absence of
demonstrable organisms, lymphomas, eosinophilia,
and lymphocytopenia all suggest an abnormal immune
system. The high incidence of infections in BB rats is
also consistent with this.

A common degenerative strain-specific lesion in BB
rats was prostatic atrophy (data not shown) character-
ized by low cuboidal glandular epithelium and the near
absence of luminal secretions. Similar changes were
not seen in Wistar rats regardless of age. Interstitial
inflammation was common in all three groups; acute
hemorrhagic prostatitis was present in one diabetic BB
rat.

Several closely related BB rats were observed to
have supradiaphragmatic accessory lobes of the liver, a
rare strain-related developmental abnormality that
has been reported in only one other strain of inbred
rat. This suggests a genetic mode of inheritance that
may be either polygenic or autosomal recessive with
low penetrance. The gross and histologic appearances
of these lesions has been described elsewhere.6

**DIABETES-RELATED LESIONS**

Table 2, part A, shows the frequency of lesions that
are related to diabetes in the BB rat and are also
strongly associated with human diabetes mellitus. One
of the most significant is insulitis, a manifestation of
the onset of JOD in man and in the BB rat. Since
insulitis is discussed in detail elsewhere in this mono-
graph, we will mention only briefly a few observations
from our study.10 The frequency of insulitis in diabetic
BB rats was inversely associated with age and duration
of diabetes, but was seen in diabetic BB rats up to
317 days old and up to 70 days post-detection. Since
insulitis was present in 16.3% of our nondiabetic BB
rats, it is apparent that insulitis does not always result
in the immediate onset of clinically apparent diabetes
mellitus. Indeed, many older nondiabetic BB rats
develop diabetes which may be a consequence of
progressive insular involvement.

**Table J.2 Frequency of Diabetes-Related Lesions (Percent)**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Diabetic BB</th>
<th>Nondiabetic BB</th>
<th>Non-BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Strongly associated with human diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulitis</td>
<td>14.0*</td>
<td>16.3</td>
<td>0</td>
</tr>
<tr>
<td>Testicular atrophy</td>
<td>30.0*</td>
<td>11.1</td>
<td>24.4</td>
</tr>
<tr>
<td>B. Weakly associated with human diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic fatty change</td>
<td>9.4</td>
<td>3.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>8.3</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Hypophagic brain damage</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
| Central pontine my-
| enolysis              | 1.1         | 0              | 0      |
| Lymphocytic thy-
| roide                 | 63.2*       | 42.1           | 0      |
| C. Not associated with human diabetes |             |                |        |
| Gastric erosions      | 32.1        | 9.7            | 0      |
| Ischaemic megaco-
| losis                 | 1.4         | 0              | 0      |

*Occurred at a younger age in diabetic BB than in other two strains.
Another lesion strongly associated with diabetes both in man and BB rats was testicular atrophy. Testicular atrophy occurred in all three groups, but occurred at much younger ages in diabetic rats. Mild testicular atrophy was observed in diabetic BB rats as early as 148 days of age, but was not apparent in nondiabetic BB or Wistar rats until one year of age. Testicular atrophy associated with diabetes and that with old age were histologically identical.

Cataracts were clinically apparent in less than 5% of diabetic BB rats but were not seen in either nondiabetic or BB Wistar rats. An increased incidence of cataracts is strongly related to hyperglycemia in man.

Several types of lesions present in BB rats are weakly associated with human diabetes (Table 2, part B). Hepatic fatty change occurred slightly more frequently in diabetic than in nondiabetic BB or Wistar rats, but in man is usually more closely associated with maturity-onset diabetes and obesity than with JOD. Acute and/or chronic pancreatitis was likewise somewhat more common in diabetic BB rats, but is only weakly associated with diabetes in man.

CNS lesions other than those due to vascular disease are uncommon in human JOD. When present, these are often iatrogenic. Two rats with hypoglycemic brain damage due to excessive insulin therapy had convulsions prior to death. Eosinophilic neurons in Somm er's sector of the hippocampus (indicative of hypoglycemic damage) were present in both rats, but eosinophilic Purkinje cells were seen in only one. A more interesting neuropathological finding was a lesion similar to central pontine myelinolysis (CPM). In man this occurs as a single focus of demyelination in the pons with relative sparing of nerve cells and axons. CPM is frequently associated with alcoholism and malnutrition and may be due to the rapid correction of hyponatremia with intravenous fluids. This is a relatively rare disorder in man and has not been previously induced in experimental animals, although it has been shown that the rapid correction of hyponatremia can cause scattered foci of demyelination in the rat. The animal in which we made this observation was an emaciated diabetic BB rat that had been treated for dehydration by multiple subcutaneous injections of saline. CPM in man has been reported in association with diabetes mellitus and fluid therapy for dehydration.

Lymphocytic thyroiditis was observed in a large proportion of diabetic BB but a smaller proportion of nondiabetic BB rats. Spontaneous thyroiditis has been reported in only one other rat strain. An association between human JOD and lymphocytic thyroiditis of other autoimmune thyroid disorders is well-established in a small population of JOD patients and has been termed "the syndrome of polyendocrine autoimmunity." The occurrence of a spontaneous lymphocytic thyroiditis in BB diabetic rats suggests that BB rats may closely mimic this syndrome of polyendocrine autoimmunity. In most instances, the infiltrates were mild and predominantly interstitial. Severe thyroiditis was strongly associated with the concurrent presence of insulitis; and as with insulitis, the prevalence of thyroiditis decreased with both age and duration of diabetes.

Table 2, part C, shows the frequency of several lesions that are diabetes-related in the rat but not associated with diabetes in man. In this category are stomach erosions and idiopathic megacolon. The most common lesions in the gastrointestinal tracts of BB rats were gastric erosions and ulcers. Gastric erosions were observed in 32.1% of the diabetic BB and 9.7% of the nondiabetic BB rats. None were seen in Wistar rats. The stomach of the rat is divided into two distinct portions by a transverse ridge—an oral portion lined by stratified squamous epithelium and a caudal portion lined by secretory glandular epithelium. Although erosions of the squamous forestomach are not uncommon in some strains of rats, mucosal lesions in the BB rat were almost exclusively confined to the glandular stomach. Stress ulcerations of this portion may be induced in rats experimentally, but they rarely occur spontaneously. We are unaware of similar reports in any other rat strain.

There were a few diabetic rats with markedly distended colons. Several of these were due to bowel obstruction caused by bowel necrosis, lymphomatous infiltrates, or prostatitis; but two of these had no physical obstruction and were termed idiopathic megacolon. Both of the latter were about 1 year old and had been diabetic more than 200 days. We examined the rectum and colon of these rats with multiple histological sections, but the ganglion cells of the myenteric plexus were present in both animals. This rules out aganglionic megacolon as a cause. Since the distended abdomen did not appear soon after birth, these ganglion cells must have been functional for some time. One possible pathogenetic mechanism is an autonomic neuropathy. This concept is supported by some studies suggesting that BB Wistar rats develop ultrastructural and functional evidence of peripheral neuropathy (received by Sima).

**NONSPECIFIC LESIONS**

Table 3 lists lesions that occurred with similar frequencies in all three groups of rats. Therefore, they are neither strain- nor diabetes-related. Hepatic infarction showed no age-related pattern, but myocardial degeneration and fibrosis was seen only in Wistar rats over 500 days old and occurred at a younger age in BB rats. Splenic extramedullary hematopoiesis was also common in all three groups. This was
associated with severe splenomegaly only in rats bearing tumors or large granulomas. Acute necrotizing vasculitis was seen occasionally in older BB rats, but this is common in older rats of many strains.25,26 Some of the older BB rats had early "chronic progressive glomerulonephropathy" (cortical cysts, glomerular basement membrane thickening, and chronic interstitial nephritis),27 a lesion common to most aged rats and often cited as the most common cause of death in some rat strains.28 Changes characteristic of diabetic glomerulosclerosis were not seen.

ORGANS VIRTUALLY FREE OF SIGNIFICANT LESIONS

Organs virtually free of significant lesions (except those mentioned above) included adrenals, aorta, bladder, brain, intestines, kidney, ovaries, parathyroid, pituitary, spleen, thymus, and uterus. Rare or incidental findings in BB rats were a pituitary chromophobe adenoma, ectopic adrenal tissue, three adrenal glands, subcutaneous orchitis, uterine strumal polyps, mammary adenocarcinoma, hepatic bile duct hyperplasia, an hepatic abscess, small-bowel necrosis, disseminated intravascular coagulation associated with pregnancy, cystitis, bronchitis, endocardial proliferation, a splenic developmental abnormality, and a subcutaneous spindle cell sarcoma.

The lesions represented in Table 2, parts A and B, support the validity of considering BB rats as a model of human JOD. However, the absence of several important sequelae of human diabetes (ie, nephropathy, atherosclerosis, and severe microangiopathy) suggests a degree of infidelity as a model for human diabetes mellitus. The lesions represented in Tables 1, 2 part C, and 3 are undesirable and uncontrollable variables that may affect experiments utilizing this model. Of these, most are minor inconveniences because of low incidences or minimal deleterious effects. Only pulmonary infections and gastric erosions are likely to be of practical consequences for most experiments.

All the disorders and frequencies in this study are characteristic of BB rats housed under the conditions present in our housing facilities. It is possible that animals housed elsewhere under different conditions may have somewhat different characteristics and incidences of diseases. This is particularly true, since several BB colonies were started from a few mating pairs of outbred rats shipped from Ottawa and were then inbred by sibling matings. This geographic isolation would tend to fix and amplify unusual traits. In addition to inbreeding, the environmental conditions at different institutions could have a significant effect on the expression of traits, even in highly inbred strains of laboratory animals.29

From these results we have drawn several general conclusions. First, the BB rat is not simply a "normal" Wistar rat that develops beta-cell necrosis with subsequent glycosuria and ketosis. Histopathological changes are consistent with there being some basic abnormalities of the immune system which have apparently been selected, along with the diabetic trait, by the process of inbreeding. It seems likely that diabetes is only a small component of a larger BB syndrome. Second, the BB rat is prone to a variety of inflammatory, degenerative, lymphoproliferative, and developmental disorders not present in outbred Wistar rats. Some of these may adversely affect experiments utilizing this model. Therefore, quality control should be assured by postmortem examination of animals involved in experiments.

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