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Spontaneous Hereditary Diabetes Mellitus in Chinese Hamster (*Cricetulus griseus*). 1. Pathological Findings.*† (24786)

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Spontaneous diabetes mellitus has been reported in a number of nonrodent species, including cattle, horses, pigs, sheep, dogs and cats(1). Estimates of relative frequency of this type of diabetes in domesticated animals vary considerably, the generally accepted figure is approximately 1:1000(1). The reported incidence for dogs ranges from 1:260 to 1:800, and for cats approximately 1:1500. The disease occurs in all age groups of animals; however, in dogs there is a marked peak at upward of 8 years. The majority of diabetic dogs are females, especially neutered females, whereas in cats, diabetes is more common in males(2-4). Microscopic findings most frequently observed in animals are "hydropic degeneration" of the islets, hyaline and amyloid changes. Diminution in number and size of islet tissue, and even complete absence were also described. In dogs, diabetes mellitus is less frequently a disease of islet tissue proper than a probable sequel to acute pancreatitis common in obese animals(2). Reports of spontaneous diabetes in laboratory rodents appear to be lacking. The literature concerning experimental surgical and drug-induced diabetes, however, is extensive(4). The present paper describes clinico-pathological findings in spontaneous diabetes mellitus of Chinese hamsters (*Cricetulus griseus*) maintained at this institution(5). The evidence indicates that it is hereditary. The genetic and hereditary aspects will be the subject of a separate report. In addition, preliminary data on chemotherapy, screening of hypoglycemic agents and metabolic studies in these hamsters will be published elsewhere.

Symptoms. Abnormal signs are usually observed from 18 days of age and later develop into either mild or severe cases. Both sexes seemed affected with about equal frequency. Diabetic hamsters exude a peculiar pungent body odor; the fur is damp and the abdomen stained yellow and soiled with urine. Some hamsters excreted up to 50-70 ml of urine in 24 hours, reflecting severe polyuria. Metabolic studies of fluid intake, urine excretion and insulin assays are in progress. At onset of symptoms, the hamsters gain considerable weight, become sluggish and severely dehydrated. Blindness is noted occasionally, the pupils turning gray, or the cornea becoming opaque. The mortality in untreated animals is extremely high. Sudden death may result from the mildest stress, such as transfer of animals from one cage to another, or by slight changes in room temperature (5-8°F). *Laboratory findings.* Clinical biochemical investigations were originally restricted to basic diagnostic determinations such as blood and urine sugar, NPN, hematocrit, urine ketone bodies, and specific gravity. Blood sugar levels, for normal hamsters, ranged 103 to 116 mg %, averaging 110 ± 6 mg %. Diabetic hamsters had levels between 200 and 600 mg %. An isolated urine sugar value in one animal was over 828 mg %. Specific gravity of urine was

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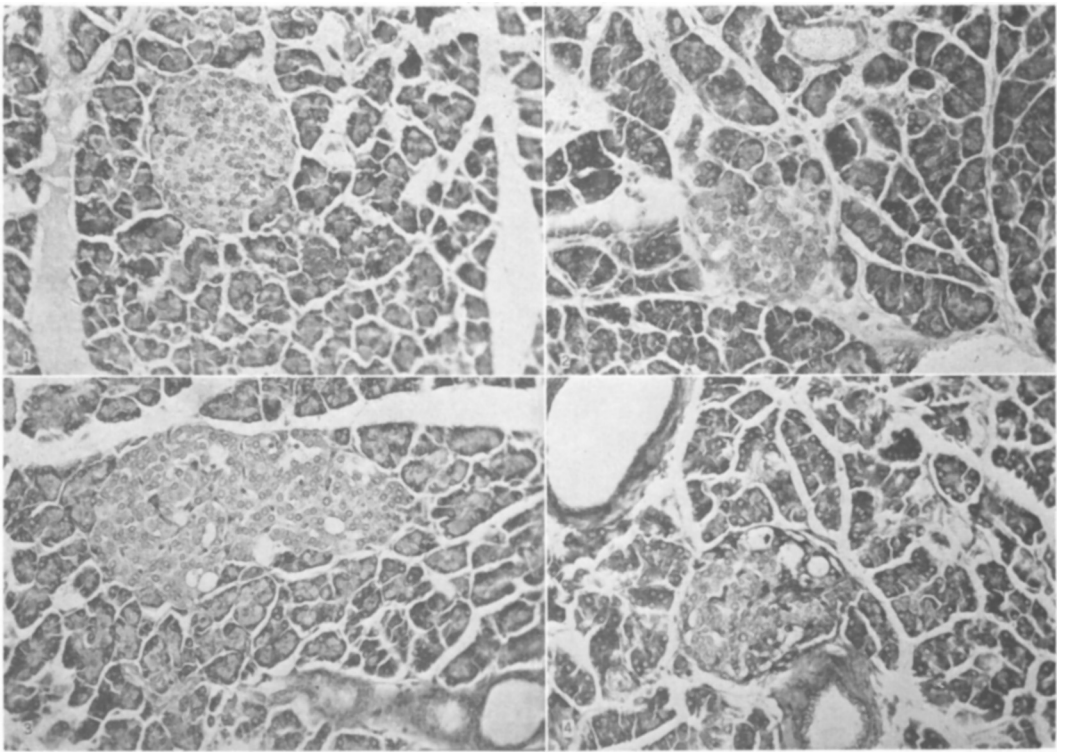

FIG. 1. Pancreatic islet of normal Chinese hamster. The islet cells form compact agglomeration with faintly stained eosinophilic cytoplasm and round nuclei of uniform type. Hematoxylin and eosin. ×163.

FIG. 2. Early changes in islet of diabetic hamster involving beta cells. Many beta cells are ballooned, their cytoplasm being unstained, cloudy, or translucent from degranulation and "hydropic degeneration." Chrome alum hematoxylin-phloxin. ×163.

FIG. 3. Severe changes of pancreatic islet in diabetic hamster. Beta cells have completely disappeared, giving to the islet a "glandular" appearance. Some of the beta cells seemed to be replaced by fat cells ("Vakat-Fett"). Chrome alum hematoxylin-phloxin. ×163.

FIG. 4. Some of the vacuolated beta cells revealed PAS-positive staining material peripherally or around karyolytic nuclei. PAS stain. ×163.

greatly elevated in the glycosuric animals, from normal 1018 to 1029+. A few severely diabetic hamsters showed signs of uremia; blood NPN levels as high as 118 mg % were found, the normal being 45 ± 3 mg %. In attempts to determine whether or not an animal was diabetic, rapid qualitative or semiquantitative tests were conducted. The most convenient methods were—Clinistix‡ for urine sugar estimates, and Acetest‡ for ketone bodies. These proved especially helpful in early detection of diabetes, in evaluating hypoglycemic therapy and in establishing effective drug dosages. *Breeding.* As the number of diabetic hamsters increased (at present more than 150 animals) it was noted that certain of our inbred lines (JFY, VSY, ORY, and JBY) were affected with a higher incidence and greater severity. An extensive breeding program has been inaugurated to evaluate the genetic background of diabetes. Because many animals are totally dependent upon diabetic therapy for continued existence, maintenance of fertility is an ever-present obstacle. Abortions, incomplete pregnancies and death at delivery were encountered. Repeated daily mating tests and improved treatment have improved the number of pregnancies and successful deliveries. Attention is also paid to diet of hamsters. All animals are maintained on Purina laboratory chow with supplementary wheat germ flakes. A detailed account will be presented later.

‡ Ames Co., Elkhart, Ind.

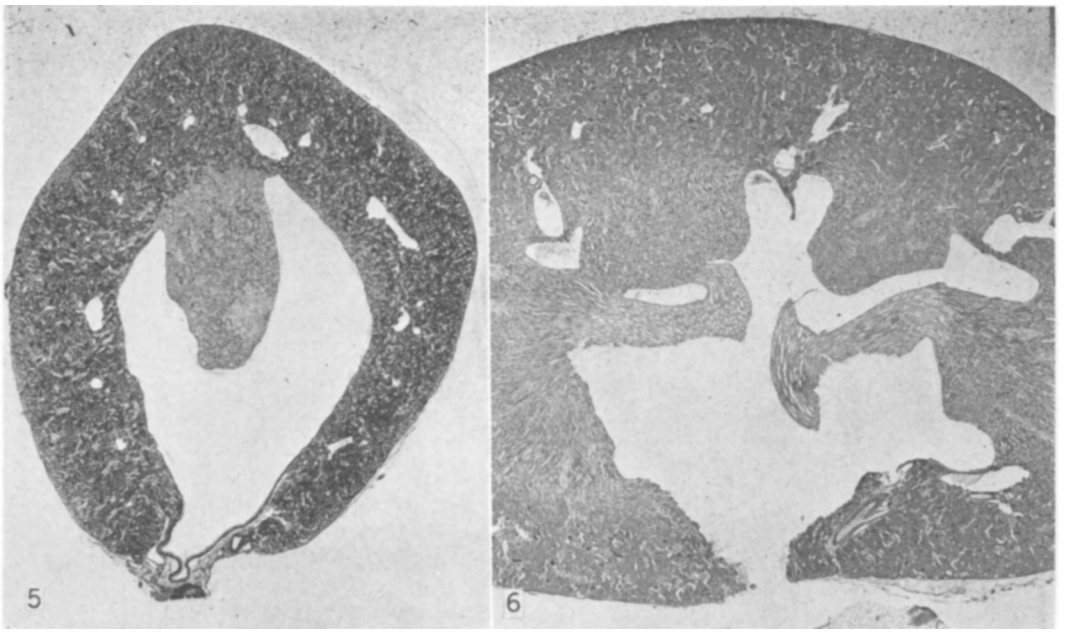

FIG. 5 and 6. Cross and sagittal section through a kidney of a diabetic hamster, showing moderate hydronephrosis. Hematoxylin and eosin. ×15.

Pathology. Macroscopic findings were restricted to the urinary system. In a few animals, pelves and recesses of kidneys in the diabetic hamsters were bilaterally distended (hydronephrosis) and retained a clear, transparent, odoriferous urine. Renal parenchyma appeared normal. The bladder was dilated and contained up to 0.5 ml urine. The pancreas was normal grossly. The liver was, in some instances, moderately enlarged and the color varied from clay to yellow. Spleen, alimentary tract, lungs, cardiovascular system and external genital organs were normal. Conjunctivitis and partial alopecia were observed. Other organs did not vary from those of normal controls Microscopic sections were routinely stained with hematoxylin and eosin; special stains employed were chrome alum hematoxylin-phloxin for the pancreas (to differentiate between islet cells) and PAS for pancreas, liver and kidneys. Tissues were fixed in Bouin's solution and cut at 8 μ, except for frozen sections which were fixed in neutral buffered formalin, cut at 16 μ, and stained with Sudan-hematoxylin.

Pancreas. Pancreatic islet changes were most striking as compared to normal islets (Fig. 1). There appeared to be comparatively few islets in diabetic animals and essentially all of them revealed abnormal cellular composition, varying in degree. Many cells were ballooned, the cytoplasm being unstained and cloudy or translucent (Fig. 2). The nucleus was either pushed peripherally and flattened, or remained in central position. It was represented by a karyolytic remnant. Many cells seemed to have disappeared from the islets, with a resultant gland-like appearance (Fig. 3). Adjacent cells appeared normal; some were crushed through expansion of the degenerating cells. All stages from an early loss of cytoplasmic staining affinity and vacuolization, to cell death with shedding of the nucleus could be observed in several islets. In normal hamsters, the islet cells formed compact clumps with faintly stained eosinophilic cytoplasm and round nuclei of uniform sizes. Alpha and beta cells were clearly distinguished in the specially stained sections. In normal hamsters, beta cells prevailed; occasional alpha cells appeared peripherally and in well vascularized islets. Diabetic hamsters disclosed low ratios; beta cells were considerably reduced and alpha cells accumulated peripherally to the islets. Many contained clusters of bright red cytoplasmic granules, espe-

cially in diabetic animals. The cytoplasm of beta cells stained only lightly basophilic, with some evidence of diffuse fine granulation. In diabetic hamsters, however, only a few appeared normal, in most islets. Their cytoplasm stained irregularly because of vacuolar changes; the fine granulation was almost indiscernible; staining affinity was entirely lacking in ballooned cells. Some of the vacuolated islet cells revealed PAS-positive staining material in the cytoplasm (Fig. 4), which accumulated around centrally located dead nuclei. Frozen sections of pancreas were extremely difficult to obtain. In most of them, the islets had "dropped out." Although it would appear that beta cells were, in some severe cases, replaced by fat cells ("Vakat-Fett") no positive fat staining was observed in the milder cases of diabetes.

Kidneys. In many hamsters there was severe dilatation of pelves and calyces, in the absence of mucosal inflammatory changes. Renal parenchyma adjacent to pelvis was not compressed (Fig. 5 and 6). Minute surface irregularities could be observed above collapsed convoluted tubules. A few scattered terminal tubules were also dilated. These contained abundant protein precipitate. The tubular epithelium was often swollen with clear cytoplasm. Many proximal convoluted tubules were collapsed; others were without lumens because of intraluminal proliferation and budding, simulating pseudoglomerular structures. Almost all glomeruli were hypocellular and showed marked intercapillary sclerosis (Fig. 7). Some glomerular tufts were deprived of endothelium, and others showed endothelial swelling. Bowman's capsule appeared slightly thickened and, in a few, glomerular tufts adhered to Bowman's capsule. PAS-positive material accumulated within the capillary tufts, completely obliterating the glomerular structure, and also in the basement membrane (Fig. 8). These depositions occurred throughout all glomeruli. Glycogen, or possibly lipoprotein deposition, was noted in the brush borders of proximal convoluted tubules and occasionally in collecting tubules. Fatty changes of tubules were very mild, but sudanophilia was essentially lacking in most kidney sections.

Liver. The lobular architecture was well preserved. Nuclei of hepatic cells displayed occasional enlargement and a "glassy" appearance suggesting increased glycogen content. In some sections there was extensive vacuolization, especially centrolobular, but occasionally involving entire lobules. Strikingly perinuclear halos were observed; these were positive for glycogen, but negative for fat. Intracytoplasmic clumping of PAS-positive material occurred diffusely in hepatic cells with no apparent zonal distribution (Fig. 9). The fat content was essentially identical with that in normal animals or was decreased (Fig. 10).

Other tissues. All organs, including skin, eyes, and pituitary, appeared to be normal. In no material examined microscopically was there evidence of retinopathy. Interestingly enough, the *pericardiac adipose tissue* revealed large quantities of PAS-positive material to an extent similar to the liver. Changes in the cardiovascular system, if present, were comparable to those in normal animals. There did not seem to be any correlation between diabetes and cardiovascular disease.

Discussion. Apart from the fact that spontaneous diabetes mellitus would be unusual in the Chinese hamster, the similarity of pathological findings to certain human changes and diabetes in experimental animals is of particular interest. The main cause of diabetes in the Chinese hamster is a disease of beta cells in the pancreatic islets; pathological changes consist of severe damage, vacuolar ("hydropic") degeneration and deficiency. These lesions have been reported in almost every type of diabetes, both human and experimental.

The problem and significance of "hydropic degeneration" has recently been reevaluated (4). The findings in the Chinese hamster lend support to the new concept that "hydropic degeneration" is a regressive response to excessive functional activity, causing degranulation and glycogen infiltration as a component of generally increased glycogen deposition late in the disease. The latter re-

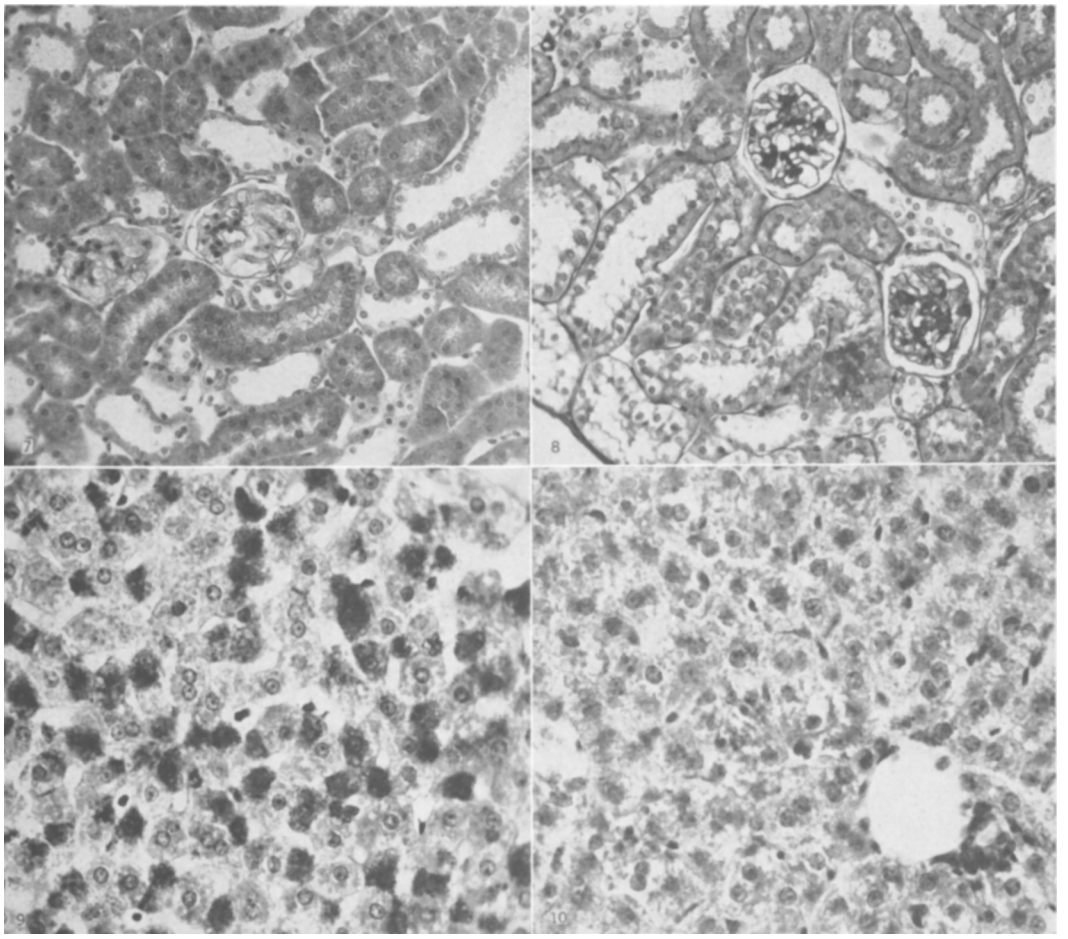

FIG. 7. Almost all glomeruli in diabetic hamsters are hypocellular and ischemic. Scattered distal convoluted tubules are dilated; others are lumenless from hypertrophy of the lining cells. Hematoxylin and eosin. ×93.

FIG. 8. Intercapillary glomerulosclerosis associated with deposition of PAS positive material. PAS. ×185.

FIG. 9. Extensive glycogen deposition occurs throughout the liver. PAS stain. ×313.

FIG. 10. Extensive vacuolization of the liver, both centrolobular and involving entire lobules. Only minute amount of fat is revealed. Sudan-hematoxylin. ×313.

sponse is analogous to the pathogenesis of glycogen nephrosis leading, in hamsters, to polyuria and uremia.

Experimentally, diabetes mellitus is produced by a variety of procedures such as administration of crude anterior pituitary extracts, adrenocortical steroids, thyroid extract, prolonged dextrose administration, alloxan and partial pancreatectomy. Morphological evolution for development of diabetes is degranulation, vacuolization, and eventual disappearance of beta cells. This sequence of events is reflected in the pancreas of diabetic hamsters suffering from varying degrees of hyperglycemia. Hyperglycemia *per se* also is a pathogenetic factor responsible for beta cell degeneration and glycogen infiltration.

In addition to morphological alterations, particular significance attaches to the problem of the etiology in diabetes mellitus. For man, 3 causes are generally listed: heredity, obesity and hormonal disturbances(6). The importance of hereditary factors in development of diabetes is stressed by many investigators. It was postulated that in man, diabetes mellitus is probably not homogeneous

genetically, and that the insulin-sensitive form of early onset behaves as if it were due to one or more recessive genes with or without reduced penetrance(7). Considerable evidence has accumulated to indicate that in the Chinese hamster, the disease is hereditary. The mode of inheritance is being investigated. It is obvious that there is tremendous value in having at hand an experimental animal in which genetic studies of diabetes can readily be made. Such a tool is also of importance in screening and study of potentially hypoglycemic agents.

Summary. Spontaneous diabetes mellitus, as observed in Chinese hamsters, has been described and compared with diabetes in human and experimental animals. Evidence was cited that in Chinese hamsters, the disease is genetically determined. Certain inferences were drawn as to the importance and significance of this observation for the future study of inheritance of diabetes, screening of hypoglycemic agents in the Chinese hamster, and metabolic studies.

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Serotonin (5-Hydroxytryptamine) Content of Canine Mastocytoma.* (24787)

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A number of biological materials have been associated with mast cells. Considerable amounts of heparin and histamine are produced and stored in dog mastocytomas(1); in addition, production of hyaluronic acid has been observed(2). However, the quantities of heparin and histamine, in dog mastocytomas are only half those of mouse mastocytomas (3). The serotonin content of dog mast cell tumors has not previously been established.

The results of our assays are reported in this paper.

Experimental. A. *Biochemical studies.* 5-Hydroxytryptamine (5-HT) was determined in 8 canine mastocytomas, following the method of Udenfriend *et al.*(4). The 5-HT equivalents found are presented in tabular form (Table I).

B. *Histochemical studies.* Sections of tumors were stained with hematoxylin-eosin, Giemsa, and toluidine blue, and by the post-coupled benzilidin reaction.† The mast cells of the different tumors contained a varying number of granules which stained deep blue with Giemsa and toluidine blue. Size of the granules decreased with malignancy of the tumors. The post-coupled benzilidin reaction is group-specific for all indol bodies, but does not identify serotonin as such(5). By this technic, the cytoplasm of the mast cells revealed a diffuse bluish staining and the granules showed a more intense blue color. A similar preparation from a mouse mastocytoma showed the same type of reaction, in regard to both cytoplasmic and granular staining (Figs. 1 and 2).

Discussion. Whereas heparin and histamine

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