Clinical diabetes due to excessive adrenocortical function or over-dosage with cortisone or ACTH is a well recognized phenomenon (1-3). Experimentally, a temporary steroid diabetes was first produced in rats by compound B and F and corticotrophin (4). This type of diabetes characteristically showed, on a constant dosage, a gradual development of glycosuria which then gradually declined to become reestablished on increasing the dosage. This adaptation of the intact animal to the administration of the diabetogenic agent is classically also observed in the diabetes produced in dogs by anterior pituitary extracts (APE) (5,6). The morphologic sequence of events in the pancreas which might account for this phenomenon of adaptation has been worked out for hypophyseal but not for steroid diabetes. It is considered that the initial response of the pancreas to APE is an increased functional activity of the beta cells as evidenced by degranulation and hyperplasia. This is followed by hydropic change, which, if allowed to persist, ultimately results in beta cell destruction and a permanent diabetes(7, 8). As recently as 1950, it was stated that cortisone causes no observable pathologic changes in the pancreas of the rat(9). However, in the same year, severe diabetes and hydropic change of the islets after large amounts of cortisone were reported in a single rabbit(10) and subsequently confirmed(1 1). Prolonged (diabetes has also been induced in guinea pigs by progressively increasing doses of cortisone(12). This was associated morphologically with hydropic change and islet hyperplasia. Furthermore, the pancreatic lesions persisted for some weeks after withdrawal of steroid therapy, despite remission of the glycosuria. In a more recent report on cortisone diabetes in rabbits, resistance to the diabetogenic agent was noted, but neither hydropic degeneration nor islet hyperplasia was observed(13). On the other hand, small doses of cortisone have been shown to cause islet hyperplasia and prevent the development of diabetes in the partially depancreatized rat (14). Furthermore, extreme centroacinar and islet hypertrophy and hyperplasia have been reported in monkeys after cortisone (15).

This would seem to indicate that an adaptive mechanism in the pancreas might account for the resistance of intact animals to the diabetogenic action of cortisone. The present experiments were designed to study the morphologic changes in the rabbit pancreas in an attempt to elucidate this phenomenon.

**Materials and methods.** Thirty-six rabbits of either sex, weighing between 2 and 5 kilos, were studied, of which 6 served as controls. Thirty were given cortisone acetate in saline suspension, intramuscularly. The dosage varied

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+ Cortisone acetate was supplied through the generosity of Dr. C. H. O'Donovan of the Upjohn
from 12.5 mg to 75 mg daily for periods ranging from 3 clays to 6 weeks. All animals were placed in individual metabolic cages and were allowed continuous free access to weighed amounts of Purina rabbit chow and water. Each rabbit was weighed hi-weekly. Twenty-four hour urine specimens were collected 3 times weekly in clean vessels to which toluol had been added. Urine glucose was determined by Benedict’s quantitative method (16). Specimens from pancreas were fixed in Zenker formol and stained with the Masson trichrome, the Gomori chrome-hematoxylin. (17) and the alcaldye fuchsin methods (18). In addition, glycogen was demonstrated by the Periodic Acid Schiff (19), counterstained with iron hematoxylin, orange G-aniline blue (20), and controlled by diastase digestion.

Results. All animals showed some debility due to cortisone administration and many died within 10 days to 4 weeks after initiating therapy. The rabbits with the highest initial weight seemed to tolerate the cortisone best, and showed the most marked glycosuria. Treated animals showed an initial weight gain varying from 150 to 250 g which then declined to the starting value by 10 to 20 g.

The rabbits differed markedly in the severity of the glycosuria that developed. In some, there were many days in which no urinary glucose was found. In others, it varied from a trace to 25 g daily (Fig. 1). Generally, there was a gradual appearance of a glycosuria between the 2nd and 5th day after starting treatment, that reached its maximum between the 5th and 9th days, and then gradually regressed. On increasing cortisone dosage, glycosuria was reestablished, reached a peak and subsequently declined. The greatest severity of diabetes appeared in animals with the lower cortisone dosages. Higher dosages seemed to diminish the diabetogenic action, but increased morbidity and mortality.

Morphologically, the most marked change consisted of a proliferation of the intercalated ducts, including the centroacinar cells, and changes in size and shape of the islets (Fig. 3). As seen in Fig. 3, there is extensive hyperplasia and hypertrophy of the intercalated ducts, making them much more prominent. In some instances, the prominence is accentuated by the presence of brilliantly stained material in the lumen. In addition, varying degrees of periductular fibrosis occasionally accompanied this hyperplasia. These changes were distributed throughout the pancreas, bitt were more marked in some areas. The inter-lobular ducts were not involved in this process and acinar dilatation was not found. The contour of islets in treated animals became irregular (Fig. 3), and they were often divided into lobules by strands of connective tissue. On closer examination, these “Mulberry” islets appeared to be formed by the continuity of portions of proliferating ductules with islet tissue and by intra-islet ductular proliferation (Fig. 5). In many cases, there was also intermingling of some acinar with the islet and duct tissue. The proliferative changes seemed to depend more on the length of therapy than on the degree of diabetes which developed, and was not observed prior to 254 weeks after starting cortisone. In animals treated for 354 weeks or longer it was uniformly present.

In addition, the well-known lesions of experimental diabetes, namely, degranulation and increased mitotic activity of the beta cells and glycogen infiltration (hydropic change)
of ductular epithelium and of beta cells were observed. The beta cell degranulation was most prominent at early stages. At that time, numerous mitotic figures were also frequently observed in the beta cells (Fig. 4). These two phenomena occurred within 3 days after instituting therapy and were present U to 10 days, regardless of the degree of diabetes which developed. Thereafter, beta cell degranulation was present, but less uniformly. Similarly, glycogen infiltration of the small ductules and centroacinar cells was a frequent finding in animals with only very mild and short-lived glycosuria. Glycogen infiltration in the beta cells seemed to require a greater degree of diabetes of longer duration. However, occasional hydropic cells could be observed in most animals regardless of the severity of the diabetes. It is interesting that hydropic change was not observed in actively proliferating ductular tissue. In many islets there was marked glycogen infiltration of a portion of the beta cells while others appeared normal.

Discussion. In some respects, the morphologic picture in the pancreas seems to go hand in hand with the metabolic picture. The early degranulation and increased mitotic activity of the beta cells would seem to represent increased functional activity and may account for the early increasing resistance to the diabetogenic action of cortisone. The glycogen infiltration of the beta cells, which is similar to that seen in all forms of experimental diabetes (II), has previously been assumed to result from exhaustion of the beta cells due to increased functional activity (21). An alternative view that it is a concomitant of the hyperglycemia per se has also been promulgated (22). This latter view obtains support from the fact that hydropic change of ductular cells, to which no increased functional activity is attributed, is as prominent as hydropic change of the beta cells.

The proliferation of the intercalated ducts, including the centroacinar cells, and the changes in the islets might also be thought of as a response to metabolic stress. New islet tissue formation from the proliferating ducts would then explain both the marked tolerance to cortisone which may develop, and the failure to produce permanent diabetes. On the other hand, it may be that hydropic change in the ductules, which is one of the earliest and most constant findings, results in ductular obstruction and the proliferation is merely a result of this obstruction. This latter hypothesis seems to be invalidated by the fact that the ductular l prolifera tion does not occur in other forms of experimental diabetes, despite hydropic change of ductular epithelium. Similarly, this lesion cannot be attributed to hyperglycemia per se as it does not appear in other forms of diabetes.

In addition, it has been shown that general debility, uremia (23), and vit. A or B deficiency (24,25) may cause pancreatic lesions which in certain respects resemble those described after cortisone. They all differ, however, in that acinar dilatation is prominent, whereas after cortisone, it is not seen. Furthermore, in unpublished observations, chronically malnourished rabbits did not show the lesion whereas in rabbits treated with vit. A, B, and B complex, in addition to cortisone, similar proliferative changes were found. Since cortisone interferes with the normal mechanism for resistance to infection, and since some of our animals died of Pasteurella pneumonia, it might be thought that the lesion here described was a form of pancreatitis. However, in animals treated with antibiotics and showing no evidence of infection, the lesion was equally prominent.

FIG. 3 (top left). Normal rabbit pallela.9 illustrating regular contour of islets and occasional barely visible intercalated ducts. PAS, trichrome X 125.
FIG. (top right). Pancreas from rabbit treated with cortisone for 6 wk, illustrating irregularity in contour of islets and marked proliferation of ductular tissue. PAS, trichrome X 125.
FIG. 4 (bottom left). Islet from rabbit after 6 days of cortisone showing a nolotie figure in a beta cell, degranulation of the beta cells and a hydropic cell. PAS, trichrome X 370.
FIG. 5 (bottom right). A “ulberr” islet from pancreas of a rabbit treated with cortisone for 5 wk illustrating lobulated appearance with ductular tissue entering into and intermingling with islet tissue. In addition, some liydropie beta cells are present. Masson tribrone X 200.

The proliferative changes cannot, therefore, in the light of present knowledge, be considered a merely
the result of hyperglycemia or of some side action of the cortisone. It must, therefore, be postulated that cortisone, like certain other steroids(26), exerts a stimulative effect on the growth of the ductular tissue of the pancreas and probably in this way induces new formation of islet tissue. This might account for the adaptation of the rabbit to the diabetogenic action of the cortisone. However, the markedly diminished sensitivity of steroid treated animals to the action of exogenous insulin(27), leaves this in doubt.

Summary. 1. Cortisone acetate administered intramuscularly to rabbits caused the gradual appearance of glycosuria which reached a maximum at the 5th to 9th days and then declined. Increased dosage reestablished the glycosuria but it again regressed. Morphologically, the most prominent lesion observed was cluctular and centroacinar proliferation and the formation of irregularly shaped and “Mulberry” islets. In addition, numerous mitotic beta cells were observed up to 10 days after starting cortisone treatment.

Similarly, degranulation of the beta cells was most prominent at early stages. Glycogen infiltration seemed to appear first in the ductular tissue and required a greater degree and duration of diabetes before affecting the beta cells. 2. The degranulation and increased mitotic activity of the beta cells are thought to be a response to hyperglycemia as is the glycogen infiltration of ducts and beta cells. The proliferative activity on the other hand is considered to represent a more direct action of cortisone on the pancreas.


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