HYPERPLASTIC AND HYPERTROPHIC CHANGES
OF THE SMALL INTESTINE IN ALLOXAN
DIABETIC RATS

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In alloxan diabetic rats both the weight and total nitrogen content of
the small intestine increased markedly. The intestinal enlargement was
caused by increased food intake and was due mainly to hyperplasia
with some hypertrophy. The intestine of diabetic rats fed on a restricted
diet did not become larger.

There have been several reports on the relationship between changes in weight
and length of the gastro-intestinal tract and the quality or quantity of food
consumed. Addis (1) found that high-residue diets increased both weight and
length of the alimentary canal in rats. He interpreted the observed change as an
adaptation to increased work, that is work hypertrophy. Fell et al. (2, 11, 12)
showed that the alimentary canal of female rats increased progressively in weight,
size, and total nitrogen content during lactation and concluded that hypertrophy
of the alimentary canal in lactating rats fed ad libitum was entirely due to increased
food intake. Durand et al. (3) reported that in growing rats, which showed
progressively increasing food intake, the weight of the intestine per 100 g body
weight increased. Using the total DNA content of the small intestine as an index
of cell number, they concluded that growth of the small intestine is essentially due
to hyperplasia.

Jervis and Levin (4) examined the small intestine of rats with chronic alloxan-
diabetes. They found that the intestine of diabetic rats markedly increased both
in diameter and length in comparison with alloxan-injected rats that did not become
diabetic, and speculated that the enlargement of the small intestine might be a
response to the amount of nutrients ingested. Schedl and Wilson (5) carried
out a similar experiment with alloxan or streptozotocin-induced diabetic rats and
obtained almost the same conclusion. In a previous paper (6) we reported briefly

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on hypertrophic or hyperplastic change of the small intestine of diabetic rats fed \textit{ad libitum}.

The present study was to see what caused this increase in intestinal weight and whether the increase was due to hypertrophy or hyperplasia.

\section*{EXPERIMENTAL}

\textit{Animals.} Male Sprague-Dawley rats, initially weighing about 150 g, were used. Diabetes was induced as described previously \cite{6} and animals, with a plasma glucose level of more than 250 mg per 100 ml of plasma, were regarded as diabetic.

\textit{Diet.} The composition of the diet is shown in Table 1. Rats were kept in individual cages and given food and water \textit{ad libitum} for 2 and 3 weeks. In some experiments, a restricted diet, equivalent to the amount consumed by normal rats, was given to diabetic rats. Food consumption, body weight change and urinary glucose were recorded daily. Urinary glucose was estimated with Tes-Tape.

Table 1. Percentage composition of diet.

\begin{center}
\begin{tabular}{ll}
\hline
Casein & 20 \\
Corn starch & 45 \\
Sucrose & 23 \\
Oil$^a$ & 5 \\
Salt mixture$^a$ & 4 \\
Vitamin mixture$^a$ & 1 \\
Choline-HCl & 0.15 \\
Cellulose powder & 1.85 \\
\hline
\end{tabular}
\end{center}

\footnote{$^a$ Purchased from Tanabe Amino Acid Research Foundation (Osaka, Japan).}

\textit{Weight of tissues.} Blood was withdrawn from the inferior vena cava of animals under ether anesthesia. The liver, kidney, stomach, and small intestine were quickly excised and washed with saline. The total length of the small intestine from the pylorus to the end of ileum was recorded.

The liver and kidney were blotted with filter paper and weighed, while the stomach and small intestine were washed free of debris, cut open, blotted with filter paper and then weighed. The small intestine was divided into three equal lengths. The upper part was used to estimate DNA, protein, and water content and the middle part was used for histological examination.

\textit{Analyses.} The blood glucose level was determined by the Somogyi-Nelson method \cite{7}. DNA was extracted by the method of SCHMIDT, THANNAUER and SCHNEIDER \cite{8} and measured by the diphenylamine method of BURTON \cite{9}. Protein content was determined by the Biuret method \cite{10}. Water content was determined by spreading a piece of small intestine on a petri dish and measuring its weight before and after drying to constant weight at 110°C.
RESULTS

The changes in body weight and food consumption of diabetic and normal rats are shown in Fig. 1. After alloxan treatment rats consumed less food than normal and their body weight decreased for several days. Then food intake increased and body weight increased in parallel. Food intake reached a plateau of 40 to 50% more than that of normal animals, one week after alloxan treatment. As diabetic rats gained far less weight than did normal rats, their average food intake, expressed in grams per 100 g body weight was 2.0 to 2.6 times that of normal rats.

Figure 2 shows the weights of the small intestine, liver, stomach, and kidney of normal and diabetic rats expressed per rat and per 100 g body weight. In both experimental periods, the weight of the small intestine and kidney of diabetic rats were more than those of normal rats especially when expressed as wet weight per 100 g body weight. The weight of the liver in diabetic rats was less than in normal rats when expressed as the weight per rat, but the same when expressed...
as the weight per 100 g body weight. The weight of stomach per rat was the same in diabetic and normal rats, but a weight increase was apparent when the weight was expressed per 100 g body weight.

Data on the small intestine are summarized in Table 2. In diabetic rats the weight of the small intestine per rat increased in 2 and 3 weeks to 46.5% and 71.5% more than that in normal animals, respectively.

The total length of the intestine increased by 6.5% and 17.8% during the same periods, respectively.

There was no difference between normal and diabetic rats in the water and protein content per g of tissue, although the total protein content increased markedly. The total DNA content in the intestine of diabetic rats increased to 32% and 40% more than that in normal rats in 2 and 3 weeks, respectively. The ratio of protein to DNA increased slightly in diabetic rats.
The results of histological examination show that in the jejunum of diabetic rats, the villi increased markedly in height (Fig. 3).

DISCUSSION

Previously (6) we reported a hypertrophic or hyperplastic change of the small intestine of diabetic rats when fed ad libitum and this enlargement of the intestine was studied in more detail in the present work.

The weight of the intestine became more than that of normal animals 2 weeks after induction of diabetes and the increase even greater after 3 weeks, when expressed either as the weight per rat or per 100 g body weight (Table 2, Fig. 2). The length of the entire small intestine also increased by 6.5% and 17.8%, 2 and 3 weeks after induction of diabetes, respectively. This is one reason for the enlargement of the gut of diabetic rats.

JERVIS and LEVIN (4) showed that the enlargement of the small intestine of diabetic rats was due to an increase in diameter and length of the gut. SCHEDL WILSON (5) attributed the intestinal growth of diabetic rats to an increase in length of the gut and in height of the villi. These results suggested that the growth of the intestine would be mainly due to hyperplasia.

The total DNA content of diabetic rats increased markedly (Table 2), indicating an increase in the cell number, namely hyperplasia. The finding could explain an increase in dry weight of the small intestine of diabetic rats described by others (4, 5).

However, the ratio of the total protein content to total DNA content, generally used as estimate of the degree of hypertrophy or atrophy of a tissue, increased slightly in diabetic rats (Table 2), indicating some hypertrophy.

These data strongly suggest that the enlargement of the small intestine observed in diabetic rats is due to hyperplasia with some hypertrophy. This was also supported by the results of histological examinations shown in Fig. 3. Namely in the jejunum of diabetic rats, the villi increased approximately 150% in length but individual cells did not appear to be hypertrophied.
Fig. 3. Microscopic photographs of jejunum mucosae in a normal (A) and an alloxan diabetic rat (B).
A) Jejunal mucosa of a normal rat fed *ad libitum* for 3 weeks; Haematoxylin and eosin, ×30.
B) Jejunal mucosa of an alloxan diabetic rat fed *ad libitum* for 3 weeks; Haematoxylin and eosin, ×30.

It is interesting that the weight of the kidney also increased in diabetic rats. This increase might represent a response to the increased work of the kidney due to
polyuria, and further studies are required on this aspect.

The weight of the stomach and liver of diabetic rats are not appreciably different from those of normal rats.

There are several reports on the gains in weight and total nitrogen in the alimentary canal observed in lactating rats (2, 11, 12). These data indicate that the increased weight of the alimentary canal is due to hypertrophy associated with polyphagia. However, Durand et al. (3) showed that in growing rats, growth of the intestine was due to hyperplasia. The enlargement of the small intestine in diabetic rats was mainly due to hyperplasia, like that in growing rats but unlike that in lactating rats.

There are two possible causes of the enlargement of the small intestine in diabetic rats. The first is that the increase in weight is due to hyperplasia associated with polyphagia. The second is that the change is induced by insulin deficiency, not by increase in daily food consumption.

These possibilities were tested and results are shown in Fig. 4. There is a very good correlation between the intestinal weight and the daily food intake per 100 g body weight. Moreover in diabetic rats on a restricted diet the small intestine did not become larger.

Fig. 4. Correlation between the weight of the small intestine and the daily food intake of normal and alloxan diabetic rats. Open and solid circles represent normal and alloxan diabetic rats fed ad libitum for 2 weeks, respectively. Open and solid triangles represent normal and alloxan diabetic rats fed ad libitum for 3 weeks, respectively, and solid squares represent alloxan diabetic rats fed a restricted diet for 2 weeks.
These results strongly suggest that enlargement of the small intestine of diabetic rats is due to increased food intake, not to insulin deficiency.

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