STUDIES ON THE
PARATHYROIDS IN ALLOXAN DIABETIC RATS

By

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ABSTRACT

The parathyroids were studied in rats with uncontrolled alloxan diabetes of about 14 days duration unaccompanied by ketonuria. The diabetic rats exhibited a significant enlargement of the parathyroid volume as compared with nondiabetic controls. Furthermore, karyometric determinations revealed significantly larger nuclei of the parathyroid cells in the diabetic rats. The urinary excretion of calcium and phosphorus was increased and the serum phosphorus level reduced in the diabetic rats as compared with the controls, whereas no difference between the groups was observed in the serum calcium level. In the diabetic rats, marked adrenal hyperplasia combined with thymus atrophy was demonstrated, indicating increased adrenocortical activity. The increased parathyroid volume is discussed against the background of the altered metabolism of calcium and phosphorus and the increased adrenocortical activity.

The literature includes only a few experimental investigations designed to disclose the existence of the functional relations between the parathyroids and the islands of Langerhans. Thus Houssay (1936) observed histological changes in the parathyroids of dogs three days after pancreatectomy. The parathyroid cells initially exhibited vacuolization, followed by shrinkage and connective tissue proliferation. In severe, non-acidotic diabetes Houssay found a parathyroid volume reduction of unspecified magnitude. The blood calcium level of the pancreatectomized dogs fell from 10–11 mg % normally to 8.2 mg % on an average and their blood phosphorus level rose from 8.8 to 14 mg %. Insulin therapy failed to normalize either these blood levels or the histological picture. Neither starvation nor phlorizin intoxication attended by glucosuria induced
hypocalcaemia or histological parathyroid changes in animals with an intact pancreas. Mentha (1941), unlike Houssay, noted parathyroid hypertrophy in a dog with spontaneous diabetes that had set in some two years previously. He observed osseous lesions resembling osteitis fibrosa in animals with diabetes induced by pancreatectomy. Engfeldt (1950) demonstrated parathyroid enlargement in rats with diabetes following pancreatectomy. The rats were pancreatectomized at the age of two months and killed a month later. Engfeldt surmised that the parathyroid enlargement was due to stimulation of the parathyroids by a hyperphosphataemia. Sen et al. (1951) found no parathyroid changes in rabbits with diabetes induced by pancreatectomy or alloxan administration but their blood calcium levels were elevated and their blood phosphorus levels depressed.

Few reports have been published concerning parathyroid morphology in human diabetes mellitus. Kraus (1923) found parathyroid atrophy, particularly in young not insulin treated diabetics, and in most cases the histological picture featured marked infiltration of adipose tissue. Conversely, in a series of probably insulin-treated cases of diabetes mellitus. Warren & LeCompte (1952) were unable to find any characteristic parathyroid lesions.

Studies on parathyroid function in human diabetes are also few. Jansen (1924) reported normal blood calcium levels in diabetics, except those with ketonuria who showed hypocalcaemia. Horstmann (1949) found normal blood calcium levels in 101 adequately treated diabetics. Talpers & Stein (1959) reported that the tubular reabsorption of phosphorus was normal in five insulin-treated diabetics, one of whom in addition had acromegaly and another sarcoidosis.

Thus the literature on the parathyroids in both human and experimental diabetes is scanty. Most workers have studied small series and their results show no consistency.

With one exception, in which the diabetic condition was induced by alloxan administration, experimental diabetes has been induced by pancreatectomy.

The aim of the present investigation was to study the morphology of the parathyroids and to estimate the calcium and phosphorus levels of blood and urine in rats with untreated alloxan diabetes.

MATERIALS AND METHODS

The investigation was performed on male albino rats of the Wistar strain. This strain has been bred for a number of years in the Department of Pathology. As soon as they had been separated from their mothers, the young rats were put on a diet of rat bread (composition see Angerwall 1959) with small supplements of milk and vitamins A, D and E. When they were two and a half months old – that is about three weeks before the experiments commenced – the rats were put on a diet composed as follows (cf. Engfeldt 1950):
Maize flour  1500 g
Wheat germs   150 g
Wheat gluten  600 g
Castor oil     300 g
'Evomin' purum  30 g
Sodium chloride  15 g
Potassium chloride  15 g
Magnesium sulphate  15 g
Ammonium phosphate 34.5 g
Calcium carbonate  60 g
Ferric citrate      6 g

This diet contains about 5.5 IU vitamin D per gram, approximately 1% calcium and a similar amount of phosphorus. The rats were given food and water *ad libitum*. They were divided into two groups: (i) the C group = controls, and (ii) the Ax group = alloxan diabetic rats.

When the trial began, the rats in the C group were 98 ± 3 days old and those in the Ax group 97 ± 3 days. The rats in the Ax group were given alloxan in a dose of 0.1 mg per g body weight of a freshly prepared 5 per cent solution of alloxan mono-hydrate in physiological saline as a subcutaneous injection through the dorsal skin. Two or three days after the alloxan injection, the 24-hour urine was measured and its sugar content determined; the urine was also tested for the presence of ketonuria. The urine was collected in special cages designed to prevent admixture of food and faecal matter. Food was given on an external balcony on which there was room only for the anterior part of the rat's body.

The duration of the experiment — that is the time from the alloxan injection to the day on which the rat was killed — averaged 14 days. During this period the rats were weighed daily, and their food intake and urinary excretion of calcium, phosphorus and sugar determined for two or three consecutive 24-hour periods. On the last day, before noon and after three hours' starvation, a blood sample was drawn from the right jugular vein under ether anaesthesia. The rats were then killed immediately by an incision into the heart. The kidneys, thymus, thyroid with the parathyroids and adrenals were at once dissected out. The adrenals and thymus were weighed on a balance with a weight set adjusted to a tolerance of 0.1 mg and an optical scale readable to the nearest 0.05 mg. After weighing the organs were fixed in 10 per cent neutral formalin solution.

The volume of the parathyroids was estimated in the usual manner (Angervall 1959) by planimetricing the drawn outlines of the parathyroids in 10 μ serial sections stained according to Weigert's haematoxylin–van Gieson at a magnification of 83 times. All serial sections were made by the same operator on the same microtome. Similarly, the drawings were all made and planimetered by the same person. Only every fifth section was drawn and planimetered, the results being barely one per cent (coefficient of variation V = 0.9) less accurate than would have been the case if every section had been drawn and planimetered.

In addition to volumetry of the parathyroids, estimations were made of the nuclear area. The measurements in question were made by the same person on coded 10 μ sections. Using an immersion objective and a total magnification of 1285 times, the largest diameter (D₀) and the diameter (D₁) perpendicular of randomly selected, intact nuclei were measured directly with an eyepiece micrometer. The nuclear area (A) was then calculated from the formula (Hellman & Hellerström 1959):
\[ A = D_1 \times D_2 \times \frac{\pi}{4} \times k, \]

where \( k \) is a constant of such magnitude that the nuclear area is expressed in \( \mu^2 \). The number of parathyroid cell nuclei thus measured was limited to 25 because the coefficients of variation for the largest diameter and the diameter perpendicular to it were not significantly larger when 100 (\( V_{D_2} = 11.67 \) and \( V_{D_1} = 10.98 \)) cell nuclei were measured than when 25 cell nuclei were measured (\( V_{D_2} = 10.70 \) and \( V_{D_1} = 13.54 \)).

Qualitative examinations of kidneys, adrenals and thymus were made on 5 \( \mu \) sections stained with Ehrlich's haematoxylin-eosin and according to Weigert's haematoxylin – van Gieson.

**Chemical Procedures**

Duplicate chemical determinations were always made. Urinary sugar was estimated with the aid of Benedict's qualitative and quantitative tests, the amount excreted being expressed in g per 24 hours. All 24-hour urines collected were tested with Clinistix (Ames Co.) as well as with Acetest (Ames Co.) to detect the presence of ketone bodies.

Blood phosphorus was determined according to Youngburg & Youngburg (1930). Thus the blood albumin was precipitated with trichloroacetic acid and the phosphorus in the filtrate converted into phosphomolybdate acid. This was reduced by the addition of elon instead of stannous chloride as in the original method. The resulting blue colour was determined colorimetrically.

Both inorganic and organic urinary phosphorus were determined in acidified samples. The urine sample was first ashed with concentrated sulphuric acid for some hours on a sandbath at 200°–250° C, whereupon perhydrol was added and the mixture returned to the sandbath until a clear solution was obtained. The sample was then treated in the same way as the blood samples. Blood calcium was determined with the aid of Eppendorf's flame photometer, using the recommended procedure and 0.2 ml of serum for each determination. Urinary calcium was determined by EDTA titration according to Lehmann (1953).

**Statistical Methods**

Student's t-test was employed for testing differences between means. Differences between regression coefficients between the levels of regression lines were tested according to Hald (1960).

**Results**

**Body Weight.** Initial and ultimate mean body weights for the rats in the C and Ax groups are given in Table 1. Initially having a slightly higher mean body weight than the controls, the diabetic rats rapidly lost weight during the course of the experiment and ultimately weighed significantly less than the controls. The percentage weight change for the controls was \( +22.8 \pm 6.0 \) and for the alloxan diabetic rats \( -11.0 \pm 2.9 \).

**Food Intake and Sugar Excretion.** Over a 72 hour period towards the end of the experiment the mean food intake was \( 13.3 \pm 0.7 \) g per day for 9 controls and \( 20.0 \pm 0.9 \) g per day for 11 diabetic rats. During the same period
the mean body weight in the C group was 202 ± 8 g and in the Ax group 193 ± 9 g. Hence, despite having roughly equal mean body weights, the diabetic rats consumed over 40 per cent more food than the controls. The mean urinary sugar excretion of the diabetic rats was 5.1 ± 0.4 g per day and their 24-hour urine volume ranged from 60 to 120 ml. No case of ketonuria was encountered.

Calcium and Phosphorus in Blood and Urine. The mean blood calcium level was 5.1 ± 0.1 meq./l for 17 rats in the C group and 5.0 ± 0.1 meq./l for 14 rats in the Ax group. The mean blood phosphorus levels of the same controls and diabetic rats were 7.2 ± 0.3 mg % and 6.1 ± 0.2 mg % respectively (P < 0.01). Accordingly the Ax group exhibited significant hypophosphataemia as compared with the C group, whilst the two groups had similar blood calcium levels. The mean daily urinary calcium and phosphorus excretions during three consecutive 24-hour periods were determined for 3 rats in the C group and 4 in the Ax group, the control rats excreting 0.9 ± 0.3 mg calcium and 8.8 ± 2.5 mg phosphorus daily and the diabetic rats excreting 11.5 ± 1.8 mg calcium and 22.7 ± 1.4 mg phosphorus daily. The respective differences are significant (P < 0.001 and P < 0.01 respectively).

Endocrine Organs. Table 1 provides data on parathyroid volume and mean adrenal and thymus weights for the C group and the Ax group. The diabetic rats evidently exhibited significant hyperplasia of the parathyroids (P < 0.01) and adrenals (P < 0.01) as well as marked thymus atrophy (P < 0.001). Equations for the regression of organ volume or weight on initial and ultimate body weight are given in Table 2. In Figs. 1 to 6 the regressive relations between organ volume and weight and initial and ultimate body weight are given for the parathyroids, adrenals and thymus in both the C series and the Ax series. It appears that there were significant regressions of parathyroid volume, adrenal

### Table 1.

<table>
<thead>
<tr>
<th>Series</th>
<th>Initial body weight g</th>
<th>Final body weight g</th>
<th>Parathyroids mm³</th>
<th>Adrenal gland mg</th>
<th>Thymus mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>162 ± 10</td>
<td>191 ± 8</td>
<td>0.145 ± 0.008</td>
<td>38.6 ± 1.5</td>
<td>254 ± 19</td>
</tr>
<tr>
<td>Ax</td>
<td>187 ± 13</td>
<td>163 ± 9</td>
<td>0.197 ± 0.013</td>
<td>47.6 ± 2.1</td>
<td>110 ± 15</td>
</tr>
</tbody>
</table>

\[ t_{C} - t_{Ax} \]

<table>
<thead>
<tr>
<th>Initial body weight g</th>
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</tr>
</tbody>
</table>

\[ t = -1.52 \quad t = +2.32^* \quad t = -3.42^{**} \quad t = -3.49^{***} \quad t = +5.98^{****} \]

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Table 2.
Regression of endocrine organ volume or weight on initial body weight.

<table>
<thead>
<tr>
<th>Organ</th>
<th>C series</th>
<th>Ax series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Regression equation</td>
</tr>
<tr>
<td>Parathyroids</td>
<td>17</td>
<td>y = 0.061 + 0.00052 x</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>17</td>
<td>y = 24.6 + 0.0861 x</td>
</tr>
<tr>
<td>Thymus</td>
<td>17</td>
<td>y = 420 - 1.0241 x</td>
</tr>
</tbody>
</table>

Regression of endocrine organ volume or weight on final body weight.

<table>
<thead>
<tr>
<th>Organ</th>
<th>C series</th>
<th>Ax series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Regression equation</td>
</tr>
<tr>
<td>Parathyroids</td>
<td>17</td>
<td>y = 0.043 + 0.00053 x</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>17</td>
<td>y = 19.1 + 0.1019 x</td>
</tr>
<tr>
<td>Thymus</td>
<td>17</td>
<td>y = 375 - 0.6336 x</td>
</tr>
</tbody>
</table>
Fig. 1.
Regression of parathyroid volume on initial body weight.

Fig. 2.
Regression of parathyroid volume on final body weight.
Fig. 3.
Regression of adrenal gland weight on initial body weight.

Fig. 4.
Regression of adrenal gland weight on final body weight.
Fig. 5.
Regression of thymus weight on initial body weight.

Fig. 6.
Regression of thymus weight on final body weight.
weight and thymus weight on initial body weight in the C group but only of parathyroid volume and adrenal weight on initial body weight in the Ax group. Corresponding regressions on ultimate body weight were present for parathyroid volume and adrenal weight in the C group.

As the mean initial body weight of the diabetic rats was higher than that of the control, the equations for the regression of parathyroid volume on initial body weight in the two groups were compared. Since the regression coefficients, $b_c$ and $b_{Ax}$ were of the same order, the regression lines may be considered parallel. However, the two lines lie at significantly different levels ($P < 0.01$). Similarly, the corresponding regression lines for adrenal weight and thymus weight do not have significantly unequal slopes and lie at significantly different levels. Consequently, even when allowance is made for the initial body weight difference between the C group and the Ax group, the parathyroids and adrenals were significantly larger and the thymus significantly smaller in the diabetic rats than in the controls. A positive regression was demonstrated between the parathyroid volume and the adrenal weight in both the control ($P < 0.01$) and diabetic rats ($P = 0.02$).

Nuclear areas in the parathyroids were estimated in 17 rats from the C group and in 14 rats from the Ax group. The mean nuclear area in these control rats was $37.33 \pm 1.28 \mu^2$ and $41.47 \pm 1.00 \mu^2$ in the diabetic rats, the mean nuclear area thus being significantly larger in the latter ($P < 0.01$).

Histological examination of the kidneys from the two groups of rats disclosed no appreciable abnormalities. The adrenals from some of the diabetic rats exhibited broadening of the fascicular zone, which was the site of some mitotic figures, and narrowing of the glomerular zone. The atrophic thymus in the Ax group displayed connective tissue proliferation here and there in the interlobular septa. No histological changes were observed in the adrenals and thymus in the C group.

**DISCUSSION**

In the experimental rats of the present investigation, induction of alloxan diabetes was followed by a marked body weight reduction and high urinary sugar excretion but in no case by ketonuria. The greatest loss of body weight was observed during the first few days following the alloxan injection, and the body weight actually tended to increase towards the end of the experimental period. The mechanism responsible for this weight recovery would seem to be that the rats compensate for their urinary sugar losses, consequent loss of body weight, by increasing their food intake. The daily mean sugar excretion remained substantially constant throughout the experiment and the diabetic condition showed no signs of improving.

In the present study no signs of intercapillary glomerulosclerosis or other
renal lesions were found two weeks after induction of alloxan diabetes. This is in agreement with the observation by Greenberg (1962) that intercapillary glomerulosclerosis in the rat did not appear until about two months after induction of alloxan diabetes.

The most usual method of ascertaining morphologically whether an endocrine organ is hyperactive or hypoactive is to estimate its degree of hyperplasia or hypoplasia, respectively, either by weighing it or determining its volume. The diabetic rats in the present investigation had a parathyroid volume some 35 per cent greater than that of the controls. Moreover, karyometric determinations revealed that the parathyroid cells had significantly larger nuclei than those in the controls. In the large majority of cases, nuclear enlargement is an indication of altered cell function, as demonstrated by Caspersson & Holmgren (1934) and Engfeldt (1950). Benninghoff (1950) spoke of a functional nuclear oedema implying that increased cellular function corresponds morphologically to a large nucleus, but it has been demonstrated that the increase in nuclear volume is associated with an increase in the absolute amount of nuclear proteins (cf. Sandritter et al. 1959). Evidently, therefore, severe alloxan diabetes of short duration and unaccompanied by ketonuria gave rise to morphological indications of parathyroid hyperactivity in the present investigation. These findings are in agreement with Engfeldt's (1950) observations in rats with diabetes induced by pancreatectomy.

In this investigation the alloxan diabetic rats showed an approximately 10-fold increase of the urinary calcium excretion and an approximately 2.5-fold increase of the urinary phosphorus excretion. The 34 per cent increase of the food not wholly correspond to the increase in calcium and phosphorus excretion.

Pitts & Alexander (1944) reported that the tubular phosphorus reabsorption competed with that of glucose in dogs. Indeed at the very time when the tubular reabsorption of glucose rose, that of phosphorus diminished, and blockage of the glucose reabsorption by phlorizin concomitantly increased the phosphorus reabsorption.

One may assume that the increased urinary excretion of phosphorus and the accompanying hypophosphataemia depend chiefly on the diminished reabsorption of phosphorus owing to the competition between phosphorus and glucose in the tubules. The osmotic diuresis induced by the large excretion of sugar also contributes to the large losses of calcium and phosphorus in the urine. Thus one explanation for the increased parathyroid activity in the alloxan diabetic rats might be that the altered metabolism of calcium and phosphorus stimulates the parathyroids to increase their activity and this is in agreement with the hypothesis of Eger (1954). The finding of a normal serum calcium level in the alloxan diabetic rats is compatible with the assumption of secondary hyperparathyroidism.
It is well known that the function of the adrenal cortex is enhanced in severe complicating diabetes and in diabetes not controlled by insulin therapy (Sokol 1961; Saba & Hoet 1962). Adrenal hyperplasia combined with thymus atrophy constitute morphological signs of adrenocortical hyperactivity in the rat (Angervall 1959). The alloxan diabetic rats in the present investigation exhibited adrenal hyperplasia and marked thymus atrophy, as morphological signs of increased adrenocortical activity. A positive regression was observed between the parathyroids and the adrenals in the control and diabetic rats, which may mean a functional relationship between the parathyroids and the adrenals. Interestingly, Lehr & Martin (1956) have presented some evidence that excess production of parathyroid hormone is mediated through the adrenal cortex and that mineral corticoids are essential for the direct or indirect activation of the parathyroid glands.

REFERENCES

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