

Adrenal function of the rat in relation to peroral administration of xylitol: depression of aldosterone

M. M. HÄMÄLÄINEN* and K. K. MÄKINEN†

*Department of Biochemistry, Institute of Dentistry, University of Turku, Finland, and

†School of Dentistry, The University of Michigan, Ann Arbor, Michigan, USA

HÄMÄLÄINEN, M.M. & MÄKINEN, K.K. 1987. Adrenal function of the rat in relation to peroral administration of xylitol: depression of aldosterone. *Acta Physiol Scand* 130, 687-693. Received 1 September, 1986, accepted 10 March 1987. ISSN 0001-6772. Department of Biochemistry, Institute of Dentistry, University of Turku, Finland, and School of Dentistry, The University of Michigan, Ann Arbor, Michigan, USA.

The effect of peroral administration of xylitol (5% or 20% in food) on adrenal function was investigated in thirty-five Long-Evans male rats. The control rats were fed either a non-substituted stock diet or a 20% glucose diet. Glucose elevated and 20% xylitol reduced the growth of the rats ($P < 0.001$), but 5% xylitol had no effect on the body weight. The concentrations of serum glucose and lactic acid decreased in rats fed 20% and 5% xylitol, respectively, but those of insulin, glucagon, corticosterone and aldosterone were not affected. In the adrenal glands, 20% xylitol loading was associated with increased epinephrine ($P < 0.05$) and norepinephrine ($P < 0.001$), but with decreased aldosterone ($P < 0.001$) concentrations. The weights and histological picture of adrenal glands were normal. The urinary pH of xylitol-fed rats decreased significantly ($P < 0.01$). Although peroral xylitol affected the levels of aldosterone and catecholamines, a normal glucocorticoid metabolism was permitted. The reduced aldosterone levels were regarded as secondary reactions, possibly resulting from alterations in electrolyte and/or acid-base balance. The increased catecholamine synthesis may be associated with the promoting effect of xylitol on intestinal calcium absorption.

Key words: adrenal hyperplasia, aldosterone, catecholamines, corticosteroids, polyols, sugar alcohols, xylitol.

The non-cariogenic sugar substitute xylitol (Mäkinen 1978) has received attention for its postulated effects on adrenal function and morphology in experimental animals (Hunter *et al.* 1978). In earlier studies a steroidogenic action of xylitol on adrenal glands was proposed (Ohnuki 1969). This effect of xylitol was demonstrated in *in vitro* studies as an increase of corticosterone production from cholesterol during incubation of rabbit adrenal gland slices in a xylitol-containing medium (Shima *et al.* 1970). In *in vivo* studies, the steroidogenic action of xylitol administration has been shown to protect humans and rabbits from the adreno-

cortical suppression during steroid therapy (Ohnuki 1969, 1971, Grabner *et al.* 1974).

Our previous studies showed that the metabolism of glucose proceeds normally in rats fed moderate (4-5%) amounts of xylitol (Hämäläinen & Mäkinen 1981, 1982). Such amounts of xylitol are normally ingested by the animals without side effects, causing no effect on the serum corticosterone levels either. Considering the fact that the metabolism of carbohydrates is closely connected to the catecholamine and corticosterone levels in mammals, these results suggest that the adrenal function is not affected by those low levels of xylitol.

A further impetus behind this study was the need to elucidate the origin of the adrenal medullary hyperplasias that reportedly occurred in rats studied at the Huntingdon Research

Correspondence: Mauri M. Hämäläinen, Institute of Dentistry, University of Turku, SF-20520 Turku, Finland.

Centre where xylitol toxicity tests were performed with rats fed large amounts of xylitol (Hunter *et al.* 1978, Roe 1984). Neither a confirmation of these results nor a plausible chemical mechanism have been published.

MATERIALS AND METHODS

Experimental animals and their treatment. Thirty-five Long-Evans male rats, aged 16 weeks and weighing 300–350 g, were randomly divided into four groups of eight or nine animals. One group that was used as a control, was fed a 1:1 mixture (wt/wt) of food meal and tap water. The composition of the food has been previously described (Hämäläinen & Mäkinen 1983). The other three groups received the same food supplemented with 5% xylitol (two groups) or 5% glucose (one group). One of the xylitol groups was given the 5% xylitol diet throughout the 8-week experimental period. In the other 5% xylitol group and the 5% glucose group the content of xylitol or glucose in the diet was raised each week by 5% until the final 20% level was reached in the beginning of the fourth week. This procedure enabled a diarrhoea-free ingestion of high amounts of xylitol. These diets were thereafter kept unchanged during the following 4 weeks. Accordingly, the final 20% xylitol and 20% glucose levels were maintained for 5 weeks, the entire feeding period lasting 8 weeks. The 5% and 20% carbohydrate levels correspond to 3100 mg (5% xylitol) and 12,700 mg (20% glucose) or 11,600 mg (20% xylitol) daily consumption, respectively (per kg body weight). The above animals were used for chemical analyses of serum and adrenal glands. A separate group of 36 Long-Evans male rats, aged 16 weeks, was also treated with the above four experimental diets. These animals were used for a histological study of the adrenal glands.

Collection and treatment of organs. At the end of the feeding schedule the rats were anaesthetized with diethyl ether in the fed state at 09.00–10.00 h. After about 2 min narcosis the aorta and the inferior vena cava were cut and the appearing blood (5–8 ml per animal) was collected into a chilled centrifuge tube containing 50 mg NaF for preventing further lactic acid production. The time-course from the start of narcosis to the collection of blood was about 2.5 min. The adrenal glands were removed immediately, photographed and placed in liquid nitrogen within 15 s following detaching of the organ. The glands were stored in liquid nitrogen for 1 month for hormone analyses. The livers were removed and weighed. Serum was separated from blood and stored at –20 °C until analysed for various chemical compounds within 4 weeks. Aprotinin (Trasyol, 1000 IU ml⁻¹) was added to the serum samples intended for the determination of glucagon. The adrenal glands

for the histological study were cut into 6 µm sections in a cryostat. The sections were stained by the Haematoxylin–Eosin method for evaluating the morphological appearance of the adrenal glands in each feeding group. The rapid fixation of the adrenal glands in liquid nitrogen preserved the morphology of the glands excellently, making the sections fully comparable to those prepared in paraffin.

Collection of urine. Preliminary semi-quantitative experiments on urine were performed on fresh 300 µl samples immediately after collection. The samples were obtained on the eighth week at least once from each animal at 09.00–10.00 h at the fed state. The samples were tested with the Rapignost® Total-Screen A test-strip (Behringwerke AG, FRG) for urobilinogen, bilirubin, ketones, ascorbic acid, glucose, protein, blood, nitrite and pH value.

Chemical and radio-immunological analyses. Serum glucose was determined by the *o*-toluidine method (Hultman 1959). Serum lactic acid was measured by the kit from Boehringer (Boehringer-Mannheim GmbH, FRG). Serum insulin concentrations were analysed with the radio-immunoassay kit of Sorin Biomedica (Sorin Biomedica, Italy). Cross-reactivity of the insulin antiserum was 105% against rat insulin (100% against human and bovine insulin).

Serum glucagon, and serum and adrenal aldosterone were analysed with the RIA kits of Serono Diagnostics (Hypolab, SA, Switzerland). The glucagon antiserum 30K has 100% cross-reactivity against glucagon and proglucagon, but it does not cross-react significantly against enteroglucagons (Weir *et al.* 1973). Aldosterone was determined from serum after extraction with a 10-fold volume of diethyl ether and from the adrenals after extraction with 6 ml of dichloromethane. According to the manufacturer's information, the cross-reactivity of aldosterone antiserum was 0.0008% against corticosterone (100% against aldosterone) and less than 0.002% against other steroid hormones tested.

Liquid chromatographic analyses. Glucocorticoids and catecholamines were analysed by high-performance liquid chromatographic (HPLC) methods. The equipment consisted of a Varian 5000 pump system (Varian Associates Inc., USA) equipped with a Pye Unicam 4020 UV Detector (Pye Unicam Ltd, UK). The glucocorticoids were fractionated on a Spherisorb Silica Column S5W (4.6 × 250 mm). The elution was accomplished isocratically with a 9:1 mixture of chloroform and methanol at a flow rate of 1 ml min⁻¹ at 22 °C. Detection of the glucocorticoids was performed at 254 nm. Under these conditions the standards, that is, 11-deoxycorticosterone, corticosterone, cortisol and aldosterone (Sigma Chemical Co., USA), were eluted within 7 min. The catecholamines were analysed on a Vydac 401 TP cation exchange column (4.6 × 250 mm). The isocratic elution was accomplished with 0.1 M Na-citrate-acetic acid buffer,

pH 4.0, at a flow rate of 2 ml min⁻¹ and detected at 280 nm. The standard catecholamines, that is, 3,4-dihydroxyphenylalanine, norepinephrine, epinephrine, DHBA, and 3-hydroxytyramine (Sigma) were eluted within 12 min.

Extraction of glucocorticoids. The glucocorticoids were extracted from 1 ml serum samples with a six-fold volume of dichloromethane (Van den Berg *et al.* 1977) and from the adrenal glands by homogenizing (in a glass homogenizer) one gland from each animal in 6 ml of dichloromethane that contained 500 ng cortisol as an internal standard. After filtering through Whatman No. 1 filter paper, the extracts were evaporated into dryness with a stream of nitrogen at 20 °C. Prior to the injection into the liquid chromatograph, the dried samples were dissolved into 50 µl (serum) or 100 µl (adrenal gland) of the eluant.

Extraction of catecholamines. The other adrenal gland of each animal was analysed for the catecholamines. Each gland was homogenized in 1.5 ml of 0.1 M HClO₄ that contained 5 mM NaHSO₃ and 15 µg of the internal standard 3,4-dihydroxybenzylamine (DHBA) in a glass potter homogenizer. After centrifugation, the catecholamines were purified from 1 ml of the supernatant by a modification of the Al₂O₃ method of Anton & Sayre (1962), described in detail in LCEC Application Note No. 14 (Bioanalytical Systems Inc., USA).

Statistics. One-way analysis of variance and the Bonferroni-modified *t*-test were used for the evaluation of the statistical significance of the differences between the treated groups and the basal diet group. The Mann-Whitney U-test was used for the statistical analysis of urinary pH-values.

RESULTS

Animal and organ weights. The weight gains of the rats are shown in Table 1. Administration of

20% glucose increased and 20% xylitol retarded the growth rates. No statistically significant differences in the sizes of the adrenal glands and the livers between the control and treated groups were found (Table 1).

Adrenal gland histology. The appearance and the histological picture of the adrenal glands were identical in all feeding groups. The histological examinations yielded no evidence of a morphological damage of the adrenal glands. No hyperplastic or neoplastic alterations or other adrenal dismorphology, were found. The adrenal gland sections from the control and treated animals were not distinguishable on the basis of their histological picture.

Glucose metabolism. The concentrations of serum glucose and lactic acid and the glucose-regulating hormones are shown in Table 2. The glucose and lactic acid levels were significantly reduced in the 20 and 5% xylitol groups, respectively. No changes in the levels of insulin, glucagon and corticosterone were seen. The adrenal medullary hormones, epinephrine and norepinephrine which partly regulate the metabolism of glucose, showed moderately elevated values in all test groups. The glucose and the xylitol regimens had, therefore, a similar effect on the levels of these medullary hormones. Only traces of DOPA and dopamine were found in the adrenal glands.

Aldosterone metabolism. Serum and adrenal aldosterone concentrations are shown in Table 2. The adrenal aldosterone levels decreased significantly in animals fed 5 or 20% xylitol. This effect was also detectable, to a certain extent, in the serum aldosterone levels of rats fed 20% xylitol.

Table 1. Body weight gains and organ fresh weights of rats fed glucose or xylitol for 8 weeks

Organ	Diet			
	Control (n = 8)	20% Glucose (n = 9)	5% Xylitol (n = 9)	20% Xylitol (n = 9)
Body weight gain (g)	67.9 ± 14.2	99.2 ± 9.2***	62.3 ± 10.6	29.1 ± 20.2***
Liver (g)	14.6 ± 1.0	16.4 ± 1.1**	15.5 ± 0.8	13.6 ± 1.5
Liver (g kg ⁻¹)†	36.5 ± 1.7	38.7 ± 3.2	38.9 ± 2.4	37.7 ± 2.7
Adrenal gland (mg)‡	62.8 ± 4.8	60.9 ± 4.7	61.7 ± 2.4	56.6 ± 3.9*
Adrenal gland (mg kg ⁻¹)†	157 ± 15	144 ± 9*	154 ± 8	161 ± 8

The values shown are mean ± SD, number of animals studied in parentheses. Significance levels between means of control and treated animals are indicated: * *P* < 0.05, ** *P* < 0.01 and *** *P* < 0.001.

† The values shown are organ weights per animal weight.

‡ The values shown are the total weights of two organs.

Table 2. Concentrations of glucose, lactic acid, glucose-regulating hormones and aldosterone of rats fed glucose or xylitol for 8 weeks

Parameter	Diet			
	Control (n = 8)	20% Glucose (n = 9)	5% Xylitol (n = 9)	20% Xylitol (n = 9)
	Serum			
Glucose (mmol l ⁻¹)	11.0 ± 1.7	11.8 ± 1.4	10.3 ± 1.0	9.2 ± 1.6*
Lactic acid (mmol l ⁻¹)	6.4 ± 1.6	5.7 ± 1.5	4.3 ± 1.4*	4.9 ± 1.5
Insulin (mU l ⁻¹)	60.6 ± 11.7	74.3 ± 20.4	61.6 ± 14.5	55.4 ± 15.7
Glucagon (ng l ⁻¹)	293 ± 68	298 ± 71	263 ± 74	214 ± 62
Corticosterone (μmol l ⁻¹)	0.95 ± 0.33	0.85 ± 0.29	0.93 ± 0.29	0.74 ± 0.30
Aldosterone (nmol l ⁻¹)	0.62 ± 0.31	0.62 ± 0.39	0.47 ± 0.26	0.28 ± 0.30
	Adrenal gland			
Corticosterone (μmol kg ⁻¹)	83 ± 46	114 ± 53	122 ± 44	85 ± 57
Epinephrine (mmol kg ⁻¹)	3.44 ± 0.50	3.78 ± 0.37	3.89 ± 0.31*	3.99 ± 0.24*
Norepinephrine (mmol kg ⁻¹)	0.71 ± 0.10	0.80 ± 0.11	0.79 ± 0.06	0.95 ± 0.10***
Aldosterone (nmol kg ⁻¹)	116 ± 52	80 ± 33	61 ± 33	28 ± 10***

The values shown are mean ± SD, number of animals studied in parentheses. Significance levels between means of control and treated animals are indicated: **P* < 0.05 and ****P* < 0.001.

Table 3. Urinary pH values of rats fed glucose or xylitol for 8 weeks

Feeding group	pH value
Control	6.1 (5.5–7.0)
Glucose (20%)	6.2 (5.5–7.0)
Xylitol (5%)	5.4 (5.0–6.5)**
Xylitol (20%)	5.1 (5.0–5.5)**

The values shown are means and ranges. The numbers of animals was eight in the control group and nine in the treated group. The urine samples were obtained on the eighth week of the regimen. ***P* < 0.01 in Mann–Whitney's U-test. Comparisons were made with the control group.

Semi-quantitative urine tests. The semi-quantitative urine tests showed differences between groups in the urinary pH values only (Table 3); xylitol feeding was associated with decreased urinary pH.

DISCUSSION

Growth of rats. The present study shows that peroral administration of large amounts of xylitol retards the growth of Long–Evans male rats compared with the consumption of an isocaloric diet containing glucose or the basal food alone. The biochemical reason for the reduced weight

gains is most likely xylitol-induced lipolysis that decreases the amount of abdominal fat (Hämäläinen & Mäkinen 1983). In addition, the slightly reduced food intake and the partial escape of the slowly absorbed polyol from the intestine to the colon (Krishnan *et al.* 1980a) may lead to the slower weight development of xylitol-fed rats. All animals were considered to be healthy throughout of the study, however, and no enlargement of the livers, for example, was found in any of the rats.

Adrenal metabolism. The diethyl ether anaesthesia may increase the serum levels of several hormones including corticosteroids and catecholamines (Black *et al.* 1969, Cook *et al.* 1973, Göthert *et al.* 1975, Tang & Phillips 1977, Holbrook *et al.* 1980, McMurtry & Wexler 1981). Although plasma renin or ACTH may increase within 1 min ether anaesthesia (Cook *et al.* 1973, Pettinger *et al.* 1975), the time delay to the corticosterone or aldosterone responses is more than 2.5 min (Cook *et al.* 1973, McMurtry & Wexler 1981). The ether narcosis may have had, however, some effect on serum corticosterone which showed fairly high basal values (Table 2). On the other hand, the serum levels of steroids corresponded well to the adrenal levels, thus giving further evidence for the validity of the steroid analyses. Catecholamines were measured from the adrenal glands only. These glands

contain a large pool of those hormones, which were not expected to be effected by the short narcotic period. Therefore, the adrenal catecholamine concentrations can be proposed as elucidating the effects of long-term xylitol feeding better than the serum levels of catecholamines. The latter are subject to considerable variations resulting from differences in blood collection methodology (Depocas & Behrens 1977, Buhler *et al.* 1978).

The main purpose of this study was to elucidate the proposed effects of xylitol on steroidogenesis and adrenal medullary hyperplasias. Xylitol did not enhance steroidogenesis in this study: the concentrations of the adrenocortical steroids, that is, corticosterone and aldosterone, were either normal or decreased. This result is in accordance with other studies in which corticosterone levels were determined (Hämäläinen & Mäkinen 1982, Boelsterli & Zbinden 1985, Mäkinen & Hämäläinen 1985). The discrepancy between the above and the earlier studies (Ohnuki 1969, 1971, Shima *et al.* 1970, Grabner *et al.* 1974) may be caused by the differences in the administration of xylitol. In the latter studies, xylitol was usually administered intravenously. This method differs decisively from the peroral administration procedure used here. Peroral xylitol is slowly absorbed from the gut but rapidly converted to glucose in the liver (Mehnert 1976, Shaw 1978), causing a much lower blood polyol concentration than during intravenous administration of xylitol.

Concerning the reported adrenal medullary hyperplasias, it is important to emphasize the fact that our 20% xylitol regimen had no effect on the histology, appearance and weights of the adrenal glands. Thus, it was not possible to confirm the findings of adrenal medullary hyperplasias in rats observed in the life-span toxicology study of xylitol (Hunter *et al.* 1978).

Although the concentrations of the adrenal catecholamines were increased compared with the control diet group, the concentrations were still within the normal range (Table 2). This increase was not considered to be reflected in glucose insufficiency since the catecholamine concentrations of rats fed 20% xylitol were close to those of rats fed 20% glucose diet.

Recently, a possible connection between adrenal medullary hyperplasias and high calcium absorption in the rat has been proposed (Roe & Bär 1985). It was stated that prolonged ingestion

of high amounts of carbohydrates which are able to promote intestinal calcium absorption, is associated with increased medullary hyperplasias and neoplasias. It was suggested that this results from the catecholamine-releasing effect of calcium in the adrenal medulla (Rubin 1970, Perlman & Chalfie 1977). Because xylitol is one of the carbohydrates promoting calcium absorption (Hämäläinen *et al.* 1985), the observed increase in the concentration of adrenal catecholamines may be connected to this phenomenon.

The principal finding of this study was the reduction of adrenal aldosterone concentrations during xylitol loading. This finding was new and surprising; aldosterone levels during xylitol loading have not been measured previously. Full evaluation of this finding would need the determination of other aldosterone-related parameters such as plasma renin, angiotensin II, ACTH, atrial natriuretic peptide, and serum and urinary electrolytes.

Aldosterone is involved in the regulation of Na, K, H⁺ and the fluid volume of the body (Marver & Kokko 1983). The reduced levels of this hormone together with the low urine pH-values suggest that alterations in the electrolyte and acid-base balance of the rats had taken place. It has been shown that the serum or plasma levels of Na⁺, K⁺ and Cl⁻ remain normal in rats during high peroral xylitol loading (Kieckebuch *et al.* 1961, Krishnan *et al.* 1980b, Hämäläinen & Mäkinen 1986). We have observed, however, that when rats are fed with 20% polyol diets, the animals develop diuresis, obviously of osmotic nature (Hämäläinen & Mäkinen 1986). Since osmotic diuresis is able to reduce aldosterone production through the renin-angiotensin-aldosterone system, it is suggested that the depression of aldosterone found in this study can be explained in terms of the diuretic effect of xylitol in the kidneys.

The acidic urine in the xylitol-fed rats (Table 3) may indicate a metabolic acidosis that was not severe, however. Lactic acidosis can be excluded on the basis of decreased serum lactic acid values (Table 2). Acidification of urine is based on the H⁺-Na⁺ exchange concept and cellular H₂CO₃ as the source of a major fraction of the secreted protons. Acidification fails if aldosterone is strongly reduced and accentuated when excessive aldosterone is secreted. This physiological regularity would thus be in disagreement with our findings since *both* decreased aldosterone *and*

acidic urine were found. A plausible explanation is that the aldosterone level was still sufficient to maintain a proper H^+ - Na^+ exchange function of the kidneys.

Based on these results it is concluded that peroral xylitol permits normal glucocorticoid and catecholamine metabolism in the adrenal glands of the rat. Also the structure of the glands was maintained unchanged in the 8-week administration of the polyol. Xylitol, however, reduced the adrenal aldosterone concentration and caused urine acidification. These effects were clearly seen at the 20% xylitol feeding level but they were also observable at the lower (5%) level of xylitol administration. Human peroral xylitol consumption may occasionally reach such levels.

The authors wish to thank Dr Hannu Laaksonen, Department of Forensic Medicine, University of Turku, for his kind help in histopathological examinations.

REFERENCES

- ANTON, A.H. & SAYRE, D.F. 1962. The study of the factors affecting the aluminium oxidetrihydroxy-indole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138, 360-375.
- BLACK, G.W., MCARDLE, L., MCCULLOUGH, H. & UNNI, V.K.N. 1969. Circulating catecholamines and some cardiovascular, respiratory, metabolic and pupillary responses during diethyl ether anaesthesia. *Anaesthesia* 24, 169-179.
- BOELSTERLI, U.A. & ZBINDEN, G. 1985. Early biochemical and morphological changes of the rat adrenal medulla induced by xylitol. *Arch Toxicol* 57, 25-30.
- BUHLER, H.U., DA PRADA, M., HAEFELY, W. & PICOTTI, G.B. 1978. Plasma adrenaline, noradrenaline and dopamine in man and different animal species. *J Physiol (Lond)* 276, 311-320.
- COOK, D.M., KENDALL, J.W., GREER, M.A. & KRAMER, R.M. 1973. The effect of acute or chronic ether stress on plasma ACTH concentration in the rat. *Endocrinology* 93, 1019-1024.
- DEPOCAS, F. & BEHRENS, W.A. 1977. Effects of handling, decapitation, anesthesia, and surgery on plasma noradrenaline levels in the white rat. *Can J Physiol Pharmacol* 55, 212-219.
- GÖTHERT, M., BISCHOFF, D. & DREYER, C. 1975. The influence of inhalation anaesthetics on catecholamine secretion from the adrenal medulla *in vivo*. *Anaesthesist* 24, 19-26.
- GRABNER, W., PESCH, H.-J., MATZKIES, F., BICKEL, H. & BERG, G. 1974. Influence of xylitol on dexamethasone-induced adrenocortical insufficiency in rabbits. *Res Exp Med (Berlin)* 162, 133-141.
- HÄMÄLÄINEN, M.M. & MÄKINEN, K.K. 1981. Metabolism of perorally administered xylitol in rat tissues. *J Nutr* 111, 107-122.
- HÄMÄLÄINEN, M.M. & MÄKINEN, K.K. 1982. Metabolism of glucose, fructose and xylitol in normal and streptozotocin-diabetic rats. *J Nutr* 122, 1369-1378.
- HÄMÄLÄINEN, M.M. & MÄKINEN, K.K. 1983. Effect of peroral xylitol on the concentration levels of lipids and electrolytes in rat tissues. *Nutr Res* 3, 497-510.
- HÄMÄLÄINEN, M.M. & MÄKINEN, K.K. 1986. Alterations in electrolyte and iron metabolism in the rat in relation to peroral administration of galactitol, mannitol and xylitol. *J Nutr* 116, 599-609.
- HÄMÄLÄINEN, M.M., MÄKINEN, K.K., PARVIAINEN, M.T. & KOSKINEN, T. 1985. Peroral xylitol increases intestinal calcium absorption in the rat independently of vitamin D action. *Mineral Electrolyte Metab* 11, 178-181.
- HOLBROOK, M.M., DALE, S.L. & MELBY, J.C. 1980. Peripheral plasma steroid concentrations in rats sacrificed by anoxia. *J Steroid Biochem* 13, 1355-1358.
- HULTMAN, E. 1959. Rapid specific method for determination of aldosesaccharides in body fluids. *Nature* 183, 108-109.
- HUNTER, B., COLLEY, J., STREET, A.E., HEYWOOD, R., PRENTICE, D.E. & MAGNUSON, G. 1978. Xylitol tumorigenicity and toxicity study in long-term dietary administration of rats. Report of Huntingdon Research Centre, Huntingdon, UK. Hoffman-La Roche, Basel, Switzerland.
- KIECKEBUCH, W., GZIEM, W. & LAND, K. 1961. Die Verwertbarkeit von Xylit als Nahrungskohlenhydrat und seine Verträglichkeit. *Klin Wochenschr* 39, 447-448.
- KRISHNAN, R., JAMES, H.M., BAIS, R., ROFE, A.M., EDWARDS, J.B. & CONYERS, R.A.J. 1980b. Some biochemical studies on the adaptation associated with xylitol ingestion in rats. *Austr J Exp Biol Med Sci* 58, 627-638.
- KRISHNAN, R., WILKINSON, I., JOYCE, L., ROFE, A.M., BAIS, R., CONYERS, R.A.J. & EDWARDS, J.B. 1980a. The effect of dietary xylitol on the ability of rat caecal flora to metabolise xylitol. *Austr J Exp Biol Med Sci* 58, 639-652.
- MÄKINEN, K.K. 1978. Biochemical principles of the use of xylitol in medicine and nutrition with special consideration of dental aspects. *Experientia (Suppl. 30)* 1-160.
- MÄKINEN, K.K. & HÄMÄLÄINEN, M.M. 1985. Metabolic effects in rats of high oral doses of galactitol, mannitol and xylitol. *J Nutr* 115, 890-899.
- MARVER, D. & KOKKO, J.P. 1983. Renal target sites and the mechanism of action of aldosterone. *Mineral Electrolyte Metab* 9, 1-18.

- McMURTRY, J.P. & WEXLER, B.C. 1981. Hypersensitivity of spontaneously hypertensive rats (SHR) to heat, ether, and immobilization. *Endocrinology* 108, 1730-1736.
- MEHNERT, H. 1976. Zuckeraustauschstoffe in der Diabetestherapie. *Int J Vit Nutr Res* (Suppl. 15) 295-324.
- OHNUKI, M. 1969. Preventing effect of xylitol on suppression of adrenocortical function by steroid therapy. In: B.L. Horecker, K. Lang & Y. Takagi (eds) *International Symposium on Metabolism, Physiology, and Clinical Use of Pentoses and Pentitols*, pp. 334-341. Springer, Berlin.
- OHNUKI, M. 1971. Clinical study on preventing effect of xylitol on adrenocortical suppression by steroid therapy. *Z Ernährungswiss* (Suppl. 11) 71-80.
- PERLMAN, R.L. & CHALFIE, M. 1977. Catecholamine release from the adrenal medulla. *Clin Endocrinol Metab* 6, 551-576.
- PETTINGER, W.A., TANAKA, K., KEETON, K., CAMPBELL, W.B. & BROOKS, S.N. 1975. Renin release, an artifact of anesthesia and its implications in rats. *Proc Soc Exp Biol Med* 148, 625-630.
- ROE, F.J.C. 1984. Perspectives in carbohydrate toxicology with special reference to carcinogenicity. *Swed Dent J* 8, 99-111.
- ROE, F.J.C. & BÄR, A. 1985. Enzootic and epizootic adrenal medullary proliferative disease of rats: influence of dietary factors which affect calcium absorption. *Human Toxicol* 4, 27-52.
- RUBIN, R.B. 1970. The role of calcium in the release of neurotransmitter substances and hormones. *Pharmacol Rev* 22, 389-428.
- SHAW, J.H. 1978. The metabolism of the polyols and their potential for greater use as sweetening agents in foods and confections. In: J.H. Shaw & G.G. Roussos (eds) *Sweeteners and Dental Caries*, pp. 157-190. Information Retrieval Inc., Washington DC.
- SHIMA, S., MITSUNAGA, M., IMAI, S. & NAKAO, T. 1970. Effect of xylitol on steroidogenesis by rat adrenal gland. *Pharmacol Res Commun* 2, 243-249.
- TANG, F. & PHILLIPS, J.G. 1977. Pituitary-adrenal response in male rats subjected to continuous ether stress and the effect of dexamethasone treatment. *J Endocrinol* 75, 325-326.
- VAN DEN BERG, J.H.M., MOL, C.R., DEELDER, R.S. & THJISSEN J.H.H. 1977. A quantitative assay of cortisol in human plasma by high performance liquid chromatography using a selective chemically bonded stationary phase. *Clin Chim Acta* 78, 165-172.
- WEIR, G.C., TURNER, R.C. & MARTIN, D.B. 1973. Glucagon radioimmunoassay using antiserum 30K: interference by plasma. *Horm Metab Res* 5, 241-244.