MACKEN1986 621

Fd Chem. Toxic. Vol. 24, No. 3, pp. 191–200, 1986
Printed in Great Britain

0278-6915/86 $3.00 + 0.00
Pergamon Press Ltd

THREE-GENERATION REPRODUCTION STUDY OF RATS INGESTING UP TO 10% SORBITOL IN THE DIET—AND A BRIEF REVIEW OF THE TOXICOLOGICAL STATUS OF SORBITOL

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(*Received* 4 *March* 1985; *revisions received* 11 *July* 1985)

Abstract—Groups of 12 male and 24 female 5-wk-old Charles River CD®(SD) BR rats (F_0) were fed a sucrose-containing ground cereal-based diet in which 0, 2.5, 5.0 and 10.0% (w/w) sorbitol was included at the expense of sucrose. The rats were first mated after 14 wk on the diet. F_{1a} litters were born 19 wk after the start of the study and F_{1b} litters at wk 30. Groups of 12 male and 24 female F_{1b} rats were first mated when 18 wk old. They gave rise to F_{2a} litters after 3 wk and to F_{2b} litters 10 wk later. Likewise, groups of 12 male and 24 female F_{2b} rats were first mated when 18 wk old, producing F_{3a} and F_{3b} litters 3 wk and 10 wk later, respectively. F_0 rats were killed 33 wk after the start of the study, F_{1a} in wk 22, F_{1b} in wk 68, F_{2a} in wk 57, F_{2b} in wk 92 and F_{3a} in wk 96. Apart from slight reductions in food consumption in sorbitol-fed F_{1b} males and in body-weight gain in sorbitol-fed F_0, F_{1b} and F_{2b} rats of both sexes, treatment was associated with no clinically observed effects. There were no deaths attributable to treatment and no adverse effects on mating performance or pregnancy rates in the parent animals of any generation. Treatment was associated with no consistent adverse effect on any measure of reproductive performance or behaviour during gestation or lactation. No abnormal pups were observed in any generation. Not unexpectedly, caecal enlargement was consistently observed at necropsy of sorbitol-treated rats of all generations and significant rises in serum calcium were observed in F_0 males and females exposed to 10% sorbitol and in F_{1b} males exposed to either 5 or 10% sorbitol. Differences between treated and control F_{3a} rats in respect of T_3 and TSH levels were probably spurious as they followed no consistent pattern. Similarly, between-group variations in gonadal weight were considered to have no toxicological significance because they lacked consistency and were not accompanied by any histologically-evident changes. Microscopic examination of lesions from F_{1a} and F_{2a} animals, of gonads from F_{1b} and F_{2b} and of selected tissues from the F_{3a} generation revealed no changes of toxicological significance. A reduced incidence of hepatocytic swelling in 10% sorbitol-treated F_{3a} females was thought to reflect no more than the slight difference in nutritional status between these animals and the controls. No abnormalities of the adrenal medulla were seen grossly in any generation or microscopically in the high-dose and control F_{3a} rats. It is concluded that sorbitol administered in the diet to three successive generations of rats at levels up to 10% had no adverse effect on growth or reproductive performance in either sex.

INTRODUCTION

Sorbitol, a hexahydric alcohol with about half the sweetness of sucrose, occurs naturally in many plant materials, including cherries, plums, pears, apples and seaweeds.

The absorption of sorbitol from the gastro-intestinal tract is by passive diffusion and is slower than that of glucose or fructose. Sorbitol is, for the most part, metabolized on its first pass through the liver, with the consequence that only very low levels are found in systemic blood or in urine (Adcock & Gray, 1957). In the liver, sorbitol is converted first to fructose and then to glucose (Hers, 1955; Wick, Morita & Barnet, 1955). Intravenous infusion of sorbitol has been found to lead quickly to fructosaemia and later, sometimes, to glucosaemia in rabbits (Seeberg, McQuarrie & Secor, 1955). Once it is absorbed, sorbitol has approximately the same calorific value as glucose. However, incomplete absorption from the gut may effectively reduce its energy value.

Oral LD_{50} values for mice, rats and rabbits are in excess of 15 g/kg body weight (Okumura & Kojima, 1972; Tanaka & Gomi, 1972; unpublished data referred to in a report by FASEB, 1979). In several other unpublished short-term studies in rabbits, rats, mice and dogs the only consistent adverse finding has been soft stools and a laxative effect when the level of incorporation in the diet was increased too quickly

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or to too high a level. If adequate time is allowed for adaptive changes in the microflora of the lower bowel then all species tested have been found to tolerate diets containing up to 20% sorbitol without exhibiting overt laxation. Adaptation is associated with caecal enlargement in rodents. This enlargement reverses rapidly on return to a diet that does not contain sorbitol.

Caecal enlargement is a generic response to poorly absorbed sugars and other carbohydrates in rats and mice. It is seen notably in response to lactose, to other polyols such as mannitol, xylitol and lactitol and to chemically modified starches. These same carbohydrates predispose to enhanced absorption of calcium from the gut, and this, in turn, is apt to give rise to nephrocalcinosis affecting mainly the renal pelvis. Additionally, and probably secondary to their effects on calcium absorption, lactose and the various polyols mentioned above, when given to rats for prolonged periods in high dietary concentrations (e.g. 20%), cause hyperplastic and neoplastic changes in the adrenal medulla, an effect reviewed by Roe & Baer (1985). The relevant data for sorbitol in this regard are derived from a 2-yr feeding study of xylitol in rats, in which a group exposed to 20% sorbitol was included for control purposes (Hunter, Colley, Street *et al.* 1978). The induced changes recorded for 20% dietary sorbitol in this study were caecal enlargement, reduced body-weight gain up to wk 78, decreased efficiency of food utilization, increased water intake and urinary output and an increased incidence of adrenal medullary hyperplasia and a decrease in absolute thyroid weight in rats of both sexes killed at the end of the study. Increased insulin levels in females at wk 52 and 78 may have been spurious findings. In another unpublished study (Gongwer & Hubben, 1969) in which rats were fed diets containing 1, 5 or 10% sorbitol for up to 2 yr, there were no clear-cut treatment-related effects other than those mentioned above, but the study did not meet present-day standards in respect of survival to 2 yr, numbers of rats per group and extent of histopathological evaluation.

A long list of mutagenicity tests has given negative results for sorbitol. These include the Ames test (Chételât, 1980; Gallandre, 1980), the rat bone-marrow test (Stanford Research Institute, 1972), host-mediated assays with *Salmonella typhimurium* strains G46 and TA1530 in mice (Stanford Research Institute, 1972), a dominant lethal assay in mice (Stanford Research Institute, 1972) and tests in Drosophila for somatic mutation, sex-linked lethal mutation and chromosomal loss (Abbott & Bowman, 1976). The only questionable findings were in an *in vitro* cytogenetic study, in which sorbitol gave rise to chromosomal abnormalities of dubious significance, and in a host-mediated assay with *Saccharomyces cerevisiae*, in which a slight increase in mitotic recombination frequency was observed in mice exposed to 5% sorbitol in the diet (Stanford Research Institute, 1972).

Negative results have been obtained in two unpublished teratology studies in rats (Food and Drug Research Laboratories, 1972; Palmer & Bottomley, 1978a), in one unpublished study in rabbits (Hummler, 1978), in small studies in mice and hamsters (Food and Drug Research Laboratories, 1972) and in a study in the developing chick embryo (Hwang & Connors, 1974).

At its 1978 meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 1978 Meeting changed the status of sorbitol from "ADI not specified" to "temporary ADI not specified". This change was made because of concern in connection with the adrenal medullary hyperplasia and decreased absolute thyroid weight seen in rats exposed to 20% sorbitol in the 2-yr study referred to above (Hunter *et al.* 1978). In 1982, JECFA removed the temporary status because the view was taken that a diet containing as much as 20% sorbitol "produced gross dietary imbalance, which may produce metabolic imbalance". "The adrenal medullary hyperplasia produced by high dietary levels of sorbitol and certain other nutrients might occur as a physiological consequence of the stresses induced in aging rats". Earlier (JECFA, 1978 & 1980), the Committee required, *inter alia*, a multigeneration reproduction study because, in an earlier study (Palmer, Bottomley, Wight *et al.* 1978b), exposure of rats to 20% sorbitol increased the duration of gestation and decreased weight and litter size, but no lower dietary concentration had been studied. The study reported in this paper was undertaken in response to this requirement.

EXPERIMENTAL

Test material. Crystalline sorbitol 834 LF (Lot nos 5106 GO and 5050 C1) was provided by an association of sorbitol producers, which includes the following members: ICI Americas Inc., Pfizer Inc., Lonza Inc., Chemurgie, CCA Biochem bv, E. Merck, Japan Sorbitol Producers Association, Roquette Frères and Xyrofin.

Diet preparation. The basal diet, as fed to the control group, was a ground cereal-based diet containing 10% sucrose and obtained from Tekland Test Diets, Madison, WI. This basal diet consisted of 23–28% crude protein, 3–5% crude fat, 3–5% crude fibre, 1.0–1.7% calcium, 0.19–0.23% magnesium and 0.90–1.1% phosphorus. In all respects it fulfilled the nutrient requirements of rats as defined by the National Research Council (1978). It was essentially free from pesticide residues and contained only low levels of aflatoxin (< 10 ppb), nitrosamines (< 13.0 ppb, except for one batch with 104 ppb) and oestrogens (< 4.0 ppb). Sorbitol was included in the basal diet at the expense of its sucrose content. Test diets were mixed weekly and, after mixing, were refrigerated for less than 48 hr (usually only 24 hr) before being given to the animals. Food was left in the food hoppers of cages for 7 days. Diets containing 2.5, 5.0 or 10% sorbitol were prepared and given to different groups of animals in this way.

Animals. Male and female Charles River CD® (SD) BR rats (non-litter-mates) were obtained at 3 wk of age from Charles River Breeding Laboratories, Wilmington, MA. Groups of 12 males and 24 females constituted the F_0 generation in the study. The groups were constructed by the allocation of animals to four groups, using a computer-generated random number table. During the growth and holding phases of the

study, animals were housed individually in suspended stainless-steel wire-mesh cages with 'deotized' animal cage boards (DACB®, Shephard Specialty Papers, Inc., Kalamazoo, MI) as linings for the urine- and faeces-collecting pans. During the mating periods, double-sized stainless-steel cages of similar design were used. Cage boards were changed at least twice each week and animals were transferred to clean cages at least fortnightly. Pregnant rats (during wk 3 of gestation) and rats with young were housed in polycarbonate cages and provided with heat-treated hardwood chips (Beta-Chip®, North-eastern Products Corp., Warrensburg, NY). Partially demineralized water and feed were provided *ad lib.* throughout the study. All the animals were housed throughout the study in one room controlled for temperature (72 ± 3°F) and humidity (50 ± 20% relative humidity). Animals were permanently identified by metal ear tags.

Experimental design. After a 2-wk acclimatization period, feeding of the basal diet or diet containing 2.5, 5.0 or 10% sorbitol to the four groups of F_0 animals was initiated. In the cases of the two latter groups (in the F_0 animals only) the concentration was built up in 2.5% steps at weekly intervals. The plan of the whole 96-wk experiment is summarized in Fig. 1, which shows that F_0 animals, one male with two females selected at random from animals of the same group, were mated after they had been on their allocated diet for at least 100 days. F_{1a} litters were born around wk 19 of the study and F_{1b} litters around wk 30. F_{1a} litters were observed up to weaning and were then killed. After the F_{1b} litters were weaned, the F_0 parents were killed (wk 33). At the same time, 12 male and 24 female F_{1b} rats were selected at random from each diet group for breeding. The procedure followed for the F_0 generation was then repeated (exposure to the diet for at least 100 days, then mating, with F_{2a} litters being born around wk 54 and F_{2b} litters around wk 65). The F_{2a} rats were killed at wk 57. The whole procedure was then repeated yet again with the random selection of 12 male and 24 female F_{2b} rats from each diet group, their maintenance for at least 100 days on the diet and then their mating to produce F_{3a} rats. F_{1b} rats were killed in wk 68 and F_{2b} rats in wk 92. Twelve male and 12

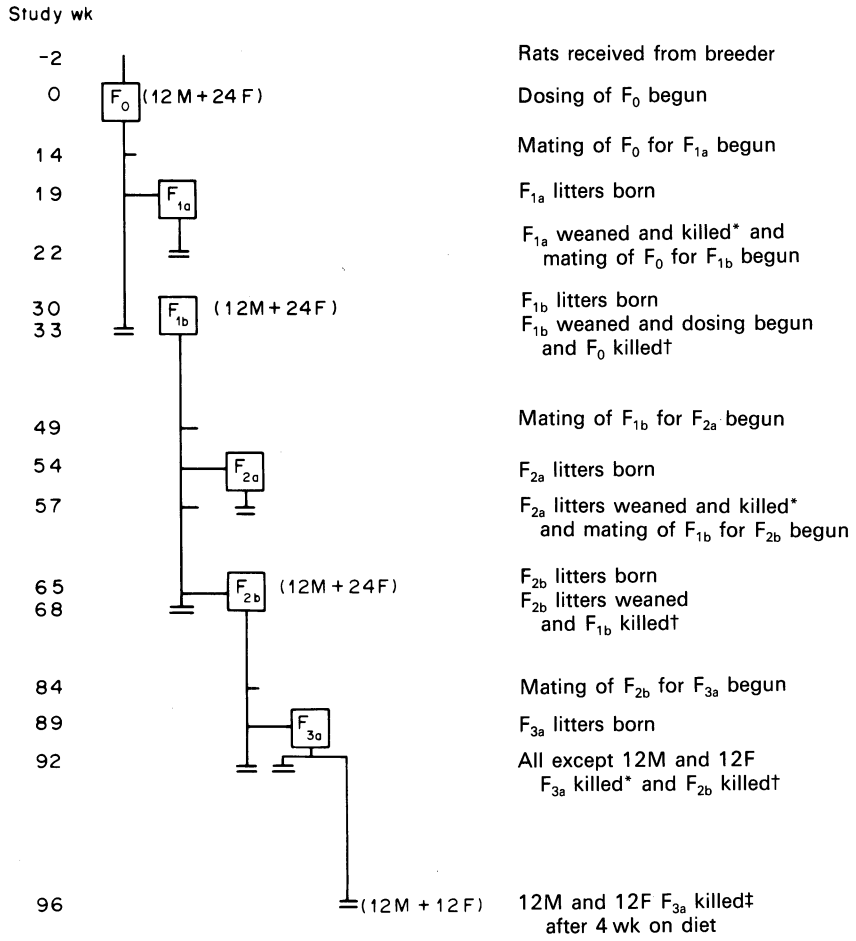

Fig. 1. Design of study: *gross examination of 2M and 2F from each of 12 litters, with weighing of gonads, spleen and caecum and microscopic examination of any lesions in these animals; †gross examination of all animals, weighing and microscopic examination of gonads, and measurement of blood-calcium in high-dose and control groups in F_0 and F_{2b} generations and in all groups of F_{1b} animals; ‡gross examination, microscopic examination of selected tissues (see text), measurement of blood-calcium and haemoglobin in all groups, and measurement of T3, T4 and TSH in high-dose and control groups.

female F_{3a} rats were killed in wk 96 after they had been on their parents' diet for 4 wk. The remaining F_{3a} rats were killed when they were weaned.

Breeding. Sibling and half-sibling matings were avoided in the pairing of F_{1b} and F_{2b} rats. Vaginal smears taken from each female daily during the mating period enabled the stage of the oestrus cycle to be ascertained. The presence of a copulatory plug or sperm in the smear was taken to indicate day 0 of gestation and the female was transferred to a single-sized cage. If no evidence of mating was obtained after 7 days, the female was placed with a different male of the same group. If, after a further 7 days, there was no evidence of mating, the female was regarded as barren. In a few animals no vaginal plug or sperm were observed despite successful mating.

Observations during gestation and lactation

Pregnant females were observed daily during gestation and weighed on days 0, 7, 14 and 20 of gestation. Females were allowed to deliver and care for their young with the minimum of disturbance. However, litters were examined on the day of delivery and the numbers of live and stillborn pups, individual live pup weights, sex ratios and gross abnormalities were recorded. After 4 days the number, sex and individual weights of pups were recorded, and pups were culled at random to achieve litters with a maximum of eight pups, four males and four females if possible. Culled pups were subjected to gross external examination and then discarded unless they exhibited grossly abnormal appearance or behaviour, in which case they were preserved intact. The numbers, sex and individual weights of pups were again recorded on day 14 and day 21 of lactation. In addition, females with litters were themselves weighed on days 0, 4, 14 and 21 of lactation.

During lactation, dams and pups were observed daily for survival and abnormalities or appearance. Pups found dead were sexed and examined for gross abnormalities. Dead pups that were less than 14 days old were discarded, but older pups were subjected to gross necropsy.

General observations on parental animals

All animals were observed daily for any overt changes in appearance or activity, or any indication of toxicity, including death. Specific notations were made regarding appearance and behaviour of dams during parturition (if observed) and lactation. Moribund animals were killed if unlikely to survive until the next observation period. These animals, and any that died on test, were subjected to a gross necropsy, and all tissues exhibiting lesions were preserved in AFA fixative (80% ethanol–40% formalin–glacial acetic acid, 90:10:5, by vol.) and processed for histopathological examination.

Body weights and feed consumption were recorded weekly during the growth phase of each generation. Following initiation of breeding procedures, males were weighed weekly and females as noted above.

Blood calcium was analysed in F_0 and F_{2b} adult animals in the control and high-level groups and in F_{1b} animals of all groups, just before they were killed, using the Worthington calcium test procedure developed by Hazleton Laboratories America, Inc. These analyses were undertaken to see whether the known effect of sorbitol on calcium absorption (see Introduction) was more or less evident in later generations than in F_0 animals.

Terminal observations

When F_0 animals were killed during wk 33 of the experiment, they were examined grossly and the gonads were weighed and examined microscopically. Sections of grossly evident lesions were prepared. Other tissues were preserved for future examination if required. The same procedure was followed with the F_{1b} adult animals that were killed in wk 68. In the case of the F_{1a} and F_{2a} animals, which were killed at weaning, two rats of each sex from each group were examined grossly and tissues were preserved for possible future sectioning and histological examination of all grossly-perceived lesions.

F_{2b} adult animals (including barren males and females) were subjected to a gross necropsy after the F_{3a} litters had been weaned. Gonads were weighed and, along with all lesions, were preserved in AFA fixative and processed for histopathological examination.

F_{3a} animals were subjected to a gross necropsy after being on test for 4 wk (post-weaning). Designated tissues were collected and preserved in AFA fixative and histopathology was performed on tissues from control and high-level group animals. Blood samples were taken from all F_{3a} animals just before they were killed, to be analysed for calcium concentration and haemoglobin content (Coulter haemoglobinomate). In view of the decrease in thyroid weight seen in a previous long-term sorbitol study in rats (Hunter *et al.* 1978), the blood samples from the F_{3a} control and high-level animals were also analysed for T3, T4 and thyroid-stimulating hormone (TSH).

Macroscopic and microscopic pathological assessment

All animals were subjected to a routine necropsy examination which included the recording of body weight, and the examination of the eyes, nose, mouth, skin and appendages. The cranium was removed and the brain, pituitary and cranial nerves were examined *in situ*. The thoracic and abdominal cavities and their contents were examined grossly following an incision in the ventral body wall.

The adrenals, brain, gonads, heart, kidneys, caecum, liver, pituitary, spleen, thymus and thyroid (with parathyroids) from all F_{3a} animals were weighed. Tissues from these organs (including three levels of the brain) and from the aorta, bone (tibia) with marrow, epididymides, oesophagus, eyes, colon, any lesions, lungs with mainstem bronchi, lymph nodes (cervical), pancreas, prostate, salivary gland (mandibular), seminal vesicles, skeletal muscle, small intestine (duodenum, ileum, jejunum), spinal cord (two levels), stomach, urinary bladder and uterus with cervix from all F_{3a} rats were fixed and, in the case of all the high-dose and control F_{3a} animals observed until wk 96, were sectioned and read routinely. In addition, the sciatic nerve, skin with mammary gland, sternum and trachea from all F_{3a} rats were fixed but not routinely sectioned.

Data analysis

Analysis of variance techniques were used to examine differences in body weight, body-weight gain, feed consumption, organ weights and clinical chemistry data. Where significant differences were indicated, Dunnett's test was performed to compare treatment with control means (Steel & Torrie, 1960). The Kruskal–Wallis test was used to compare the number of pups born, the percentage born alive, percentage survival and sex ratios (Hollander & Wolfe, 1973). Where significant differences were indicated, Dunn's test was used to compare treatment with control means (Dunn, 1964). Reproductive indices were analysed using contingency tables, for testing whether probability of successful mating or gestation was independent of the treatment group.

RESULTS

Diet analysis

Analyses of samples of each test diet taken at approximately 4-wk intervals throughout the study showed that all were within 15% of the correct percentage.

Clinical observations, feed intake and body weights

There were no clinical signs of toxicity and no deaths attributable to treatment in the F_0, F_{1b} or F_{2b} rats and the mating performance and pregnancy rates of these parent animals were not affected by treatment.

Sorbitol-treated F_{1b} males consumed less diet than did control F_{1b} males, and sporadic variations in feed consumption were seen in sorbitol-fed F_{1b} females. No effects of treatment were seen in the feed consumption patterns of the F_0 or F_{2b} rats. Reduced weight gain was recorded in response to sorbitol in both sexes (Table 1). The effect was virtually confined to the 10% level of sorbitol incorporation in the diet. It was more marked in females than in males and more marked in the F_0 generation than in the F_{1a} and F_{2b} generation. Body-weight gain of dams was unaffected by sorbitol during gestation but was significantly higher in the 10% sorbitol groups during lactation (Table 2). This finding reflected a lower body weight than that of controls on day 0 of lactation rather than a higher body weight on day 21.

Indices of fertility and reproductive performance

As shown in Table 3, exposure to sorbitol had no adverse effect on the fertility of animals of either sex. Table 4 summarizes the results of the study in terms of the mean number of days required to mate, the mean length of gestation, the mean number of pups per litter, the percentage of pups that were still-born, the percentages of pups that died between days 0 and 4 and between days 4 and 21 of lactation, the mean pup weight on day 0, and the mean weight gains over days 0–4 and days 0–21 of lactation. Treatment was without effect on days required to mate or on length of gestation in any of the five generations studied. It was also without effect on the sex ratio in any generation.

The only significant difference between treatment and control groups in mean number of pups per litter occurred in the F_{2b} generation and was apparently due to a spuriously low mean in the control group. An apparently higher percentage of stillborn pups in 10% sorbitol-fed animals of the F_{1a} and F_{1b} generations was counterbalanced by the higher levels of still-borns in controls than in treated groups in the F_{2a}, F_{2b} and F_{3a} generations. The same comment applies to the comparisons of the percentages of pups that died between day 0 and day 4 of lactation. Moreover, more control than treated F_{2a} and F_{2b} animals died during days 4–21 of lactation.

A significantly higher mean birth weight of F_{3a} pups in the 5.0% sorbitol group than in the control group is most plausibly explained by the mean for the

Table 1. Mean body weights in F_0, F_{1b} and F_{2b} generations fed 0–10% sorbitol in the diet

Time from weaning (wk)	Dietary level (%)...	Mean body weight (g)							
		Males				Females			
		0	2.5	5.0	10.0	0	2.5	5.0	10.0
		F_0 generation†							
0		165	158	160	158	135	128**	131	130
4		365	362	350	353	229	218	221	211*
8		448	455	431	439	272	256*	263	252**
15		537	538	511	513	315	296*	303	286**
0–15	Weight gain...	372	380	351	355	180	168	172	153
		F_{1b} generation							
0		170	161	170	157	142	137	139	131
4		386	382	384	349	238	235	225	229
8		494	492	478	443*	284	280	269	268
14		573	574	557	517	318	315	296*	296*
0–14	Weight gain...	403	413	387	360	176	178	157	165
		F_{2b} generation							
0		162	170	191	180	136	140	150	138
4		376	387	400	371	229	237	227	230
8		494	503	502	477	277	286	272	272
14		568	578	574	539*‡	305	321	304	302
0–14	Weight gain...	406	408	383	359	171	181	154	164

†In the F_0 generation, test diets were fed from 2 wk after weaning.
‡Difference was significant at wk 13 but not at wk 14.
Values are means for groups of 12 males and 24 females and those marked with asterisks are significantly lower than the corresponding control values: $*P < 0.05$; $**P < 0.01$.

Table 2. Mean body weights during gestation and lactation for three generations of dams fed 0–10% sorbitol in the diet

Generation	Dietary level of sorbitol (%)	No. of dams	Gestation: Mean body weight on gestation day: 0	7	14	20	Gestation: Weight change (g) on days 0–20	No. of dams†	Lactation: Mean body weight on lactation day: 0	4	14	21	Lactation: Weight change (g) on days 0–21
F_0 dams/F_{1b} offspring	0	18	335	361	387	455	+120	20	372	376	378	356	−15
	2.5	20	321	345	372	438	+116	20	359	370	364	341	−17
	5.0	17	323	348	374	441	+119	18	356	362	370	347	−9
	10.0	22	315	334	358	432	+116	22	350	361	375	361	+13**
F_{1b} dams/F_{2b} offspring	0	15	348	376	404	464	+116	17	383	388	389	371	−12
	2.5	17	359	389	420	497*	+138*	18	398	413	410	380	−18
	5.0	16	330	359	392	463	+133	18	378	390	396	370	−8
	10.0	19	328	350*	379	453	+126	21	357	378	399	368	+11**
F_{2b} dams/F_{3a} offspring	0	15	308	339	367	432	+125	16	347	348	356	339	−8
	2.5	20	318	347	375	442	+125	21	356	359	367	345	−12
	5.0	20	301	333	362	427	+126	21	347	349	361	334	−14
	10.0	20	295	321	346	413	+118	20	323	337	347	345	+22**

†The numbers of dams weighed during lactation exceeded, in some groups, the number weighed during gestation because no vaginal plugs or sperm were seen in some animals that subsequently delivered litters; as these rats were not identified as 'pregnant', their gestation body-weight data were not recorded, but they were weighed during lactation.
Values are means for the numbers of dams stated and those marked with asterisks differ significantly from the control value: *$P < 0.05$; **$P < 0.01$.

controls being spuriously low. Treatment was without effect on mean body-weight gain during days 0–4 of lactation. However, the 10% sorbitol diet was thereafter associated with slightly reduced mean weights gains in the F_{1a}, F_{1b}, F_{2a} and F_{2b} pups and the 5% diet was similarly associated with a reduction in weight gain during lactation in the F_{2a} and F_{2b} generations.

Behaviour and appearance

No abnormalities in the appearance or behaviour of females were observed during gestation or lactation and no abnormal pups were observed in any generation.

Blood analyses

A comparison of blood-calcium levels in twelve 10% sorbitol-treated F_{2b} rats of each sex and in similar numbers of the corresponding controls revealed no difference (Table 5). Similarly, no treatment-associated difference was found in F_{3a} rats of either sex. However, in the F_0 males and females and F_{1b} males, exposure to 10% sorbitol was associated with a significant elevation of blood calcium. A significant rise was also seen in F_{1b} males exposed to 5% sorbitol. Since, like other polyols and lactose, dietary sorbitol is known to increase the absorption of calcium from the gastro-intestinal tract, these observations of increased blood-calcium levels are not surprising. However, the reason why the same effect was not evident in the F_{2b} and F_{3a} generations is obscure.

Sorbitol treatment had no effect on haemoglobin values in F_{3a} rats (Table 6).

In F_{3a} generation rats of both sexes, 10% sorbitol-treatment was associated with T_3 levels that were significantly ($P < 0.01$) different from those in the corresponding controls. However, the differences were in the opposite directions in the two sexes (Table 6). T_4 levels showed no difference between high-dose and control groups in either sex. TSH levels were higher in 10% sorbitol-treated males ($P < 0.05$) but in females there was a big but not significant difference in the opposite direction. It is reasonable to conclude that these observations were spurious findings. This view is reinforced by the fact that treatment had no observed effect on the weight or gross appearance of the thyroid in any generation.

Gross necropsy and organ weights

Treatment was associated with no macroscopically evident effects in any of the parental (F_0, F_{1b}, F_{2b}) or young (F_{1a}, F_{2a}, F_{3a}) animals at necropsy, except in the caecum, spleen and gonads (Table 7). It was to be expected from previous studies that the rats fed diets containing sorbitol would exhibit enlargement of the caecum. Apparently reduced spleen weights in treated F_{1a} females may be regarded as a chance finding in view of the difference in the opposite direction in F_{3a} females.

Feeding of 10% sorbitol was associated with significantly higher mean weights of the ovary in F_0 females and of the testes in F_{2b} males. It is reasonable

Table 3. Fertility indices for male and female rats fed 0–10% sorbitol in the diet

Dietary level of sorbitol (%)	Fertility index* for generation: F_0 F_{1a} litter M	F_0 F_{1a} litter F	F_0 F_{1b} litter M	F_0 F_{1b} litter F	F_{1b} F_{2a} litter M	F_{1b} F_{2a} litter F	F_{1b} F_{2b} litter M	F_{1b} F_{2b} litter F	F_{2b} F_{3a} litter M	F_{2b} F_{3a} litter F
0	83	83	83	95	100	68	83	81	92	83
2.5	100	95	75	91	100	91	75	78	100	95
5.0	100	88	92	82	92	91	92	86	92	88
10.0	92	100	92	96	92	86	92	95	100	100

*Male fertility index: (no. of males shown to be fertile—as defined by a female giving birth to a litter/no. placed with females) × 100. Female fertility index: (no. of females producing a litter/no. mated) × 100.

to ascribe both these observations to chance since similar differences were not observed in other generations and the weight differences were not associated with microscopically detectable abnormalities.

Microscopic examination of tissues

No toxicologically significant or other changes attributable to treatment were detected in any of the pathological examinations, including the extensive histopathological assessment carried out in 12 high-dose and 12 control F_{3a} rats of each sex. A lower incidence of hepatocytic swelling in 10% sorbitol-treated F_{3a} females compared with corresponding controls may have reflected a slight difference between the groups in nutritional status. However, if so, it is noteworthy that the F_{2b} females, liver sections from which were also examined, did not show this difference. The incidence of mineral deposition in the kidneys was higher in F_{3a} control females than in F_{3a} females treated with 10% sorbitol, but this was probably a chance finding and, in any case, was of no toxicological significance.

Table 4. Effects of feeding 0–10% dietary sorbitol on various indices of reproductive performance in successive generations of rats

Parameter	Dietary level of sorbitol (%)	Reproduction data for litters of generation: F_{1a}	F_{1b}	F_{2a}	F_{2b}	F_{3a}
Mean no. of days to mate	0	3.8	3.7	4.0	4.6	4.0
	2.5	3.6	4.4	3.6	4.4	3.7
	5.0	4.0	3.0	5.2	3.1	3.1
	10.0	4.2	4.4	4.9	3.6	3.3
Mean length of gestation (days)	0	22.6	22.7	22.5	22.7	22.2
	2.5	22.5	22.4	22.2	22.4	22.4
	5.0	22.7	22.4	22.3	22.4	22.7
	10.0	22.3	22.1	22.5	22.4	22.6
Mean no. of pups/litter	0	13.2	12.4	11.3	10.8	13.6
	2.5	12.4	12.2	13.6	13.8**	12.9
	5.0	10.4	12.1	12.8	13.1*	11.1
	10.0	12.0	13.0	13.1	12.8	13.0
Pups still-born (% of total)	0	1.5	2.2	3.3	2.4	5.0
	2.5	2.5	0.7	0.7	1.6	1.6
	5.0	3.2	2.4	0.8	1.1	0.7
	10.0	8.0	5.3	1.6	0	2.0
Pups deaths on days 0–4 of lactation (%)	0	1.9	9.2	15.5	7.4	3.1
	2.5	4.9	7.3	1.9	1.3	2.4
	5.0	4.2	3.5	3.2	2.7	2.4
	10.0	16.6†	5.8	2.8	1.7	0.9
Pups deaths on days 4–21 of lactation (%)	0	0	4.0	7.7	6.8	0
	2.5	2.4	0.7	0	0	0
	5.0	2.6	2.2	0.6	2.1	0
	10.0	3.5	2.5	3.3	2.4	0.6
Mean pup weight (g) on day 0	0	6.7	6.6	6.4	6.8	6.0
	2.5	6.7	6.6	6.4	6.8	6.4
	5.0	6.8	6.6	6.5	6.6	6.8**
	10.0	6.4	6.6	6.6	6.7	6.5
Mean weight gain (g) on days 0–4	0	4.1	4.9	4.4	4.5	3.8
	2.5	4.5	4.5	4.1	4.2	4.3
	5.0	4.7	4.5	4.3	3.8	4.9
	10.0	4.0	4.0	4.2	4.3	4.4
Mean weight gain (g) on days 0–21	0	43.0	51.1	49.2	50.9	45.8
	2.5	44.1	47.2	47.3	49.9	44.3
	5.0	43.5	47.1	44.9	46.6	45.9
	10.0	40.2	45.0	45.4	46.9	43.2

†The standard deviation for this value was approximately 6, and the difference between 1.9% deaths for the controls and 16.6% deaths for the high-level exposure group was not statistically significant.

Values are means for 15–22 litters and those marked with asterisks are significantly higher than the control value: *$P < 0.05$; **$P < 0.01$.

Table 5. Effects of feeding 0–10% dietary sorbitol on blood-calcium levels in four generations of rats

Dietary level (%)	Generation...	Mean calcium level (mg/100 ml blood) F_0	F_{1b}	F_{2b}	F_{3a}
		Males			
0		9.8	9.9	9.1	10.4
2.5		—	10.0	—	10.3
5.0		—	10.2*	—	10.2
10.0		10.2**	10.3**	9.3	10.2
		Females			
0		10.0	10.0	10.0	9.9
2.5		—	10.2	—	10.0
5.0		—	10.3	—	10.0
10.0		10.4*	10.1	10.0	9.7

Values are means for groups of 12 male rats and for groups of 24 females, except for the F_{3a} groups (12) and the F_{2b} control group (22). Those marked with asterisks are significantly higher than the control value: $*P < 0.05$; $**P < 0.01$.

Adrenal medulla

This study was not designed to investigate the adrenal medullary hyperplasia seen in an earlier long-term study in which rats were exposed to 20% sorbitol in the diet (Hunter *et al.* 1978). Exposure was of too short duration for this purpose and the highest exposure level was only 10%. Nevertheless, it is noteworthy that no abnormalities were seen grossly in the adrenal glands of any generation, or microscopically in the F_{3a} animals, and there was no difference in adrenal weight between treated and control rats.

DISCUSSION

The main conclusion to be drawn from this study is that, while the expected effects on caecal size and blood calcium were recorded, exposure to 10% dietary sorbitol had no adverse effect on any aspect of reproductive function in rats.

Although some of the differences between control and treated animals of the F_{3a} generation in levels of circulating T3, T4 and TSH reached statistical significance, the results for the two sexes were contradictory and failed to constitute a toxicologically meaningful pattern. The conclusion that these findings lacked toxicological significance is supported by the absence of any evidence of an effect of treatment on the weight or gross appearance of the thyroid in any generation. These negative findings suggest that the decrease in thyroid weight recorded in rats fed sorbitol for 2 yr in the study reported by Hunter *et al.* (1978) was probably a chance finding without toxicological significance.

The increase in caecal weight observed in the study was expected in the light of metabolic data on sorbitol and the results of previous studies. On the other hand it is reasonable to attribute to chance the apparent effects of treatment on gonadal weight and spleen weight in view of their inconsistency and the absence of any histological evidence of pathological change.

In view of the known effect of dietary sorbitol in increasing calcium absorption from the gastrointestinal tract in rats (Roe & Baer, 1985) it was not surprising that significantly raised calcium levels were recorded for F_0 (both sexes) and F_{1b} (males only) animals fed on 10% (and in the latter group 5%) sorbitol. However, it is interesting that similar differences between the control and treated groups were not seen in F_{2b} and F_{3a} animals. Moreover, no pathological changes secondary to hypercalcaemia were seen in rats of either sex in any generation.

Table 6. Haemoglobin values and circulating T3, T4 and TSH concentrations in F_{3a} generation rats fed from the F_0 generation on 0–10% dietary sorbitol

Dietary level (%)	Mean values for: Haemoglobin† (g/100 ml)	T_3 (ng/100 ml)	T_4 (μg/100 ml)	TSH (ng/ml)
		Males		
0	14.4	64	5.6	1499
10	14.4	74**	6.3	2341*
		Females		
0	15.1	86	4.9	994
10	14.7	71**	4.3	647

T_3 = L-3,5,3′-Triiodothyronine T_4 = L-Thyroxine
TSH = Thyroid-stimulating hormone (thyrotropin)

†Haemoglobin levels determined in the intermediate dosage groups were 13.7 and 13.8 g/100 ml for males and 14.5 and 14.2 g/100 ml for females in the 2.5 and 5% sorbitol groups, respectively.

Values are all means for groups of 12 rats and those marked with asterisks differ significantly from the control value: $*P < 0.05$; $**P < 0.01$.

Table 7. Effect of multigeneration feeding of 0–10% dietary sorbitol on the weights of gonads, spleen and caecum of rats

Sex	Dietary level of sorbitol (%)	Mean organ weight (g) and (in brackets) relative organ weight (g/100 g body weight) for:											
		F_0 Adults		F_{1a} Weanlings		F_{1b} Adults		F_{2a} Weanlings		F_{2b} Adults		F_{3a} Adults	
		Gonads											
M	0.0	3.63	(0.58)	0.27	(0.50)	3.81	(0.59)	0.30	(0.51)	3.65	(0.60)2.99		(0.91)
	2.5	3.62	(0.58)	0.26	(0.50)	3.92	(0.59)	0.29	(0.51)	3.69	(0.61)	2.82	(0.86)
	5.0	3.53	(0.58)	0.23	(0.48)	3.75	(0.59)	0.28	(0.52)	3.68	(0.62)	2.91	(0.90)
	10.0	2.45	(0.58)	0.26	(0.54)	4.02	(0.66)	0.28	(0.52)	4.05*	(0.71)**	3.16	(0.97)
F	0.0	0.12	(0.034)	0.03	(0.063)	0.13	(0.034)	0.04	(0.071)	0.13	(0.039)	0.10	(0.049)
	2.5	0.12	(0.035)	0.04	(0.090)	0.14	(0.035)	0.04	(0.078)	0.15	(0.043)	0.10	(0.047)
	5.0	0.12	(0.035)	0.03	(0.071)	0.12	(0.032)	0.04	(0.080)	0.15	(0.044)	0.10	(0.045)
	10.0	0.15**	(0.044)**	0.03	(0.068)	0.13	(0.037)	0.03	(0.064)	0.15	(0.046)	0.10	(0.046)
						Spleen							
M	0.0			0.27	(0.49)			0.32	(0.56)			0.79	(0.24)
	2.5			0.24	(0.45)			0.32	(0.56)			0.85	(0.26)
	5.0			0.23	(0.47)			0.32	(0.59)			0.82	(0.25)
	10.0			0.22	(0.46)			0.30	(0.57)			0.78	(0.24)
F	0.0			0.26	(0.50)			0.31	(0.61)			0.54	(0.26)
	2.5			0.22*	(0.44)			0.30	(0.56)			0.57	(0.26)
	5.0			0.24	(0.50)			0.30	(0.59)			0.64*	(0.30)
	10.0			0.20**	(0.44)			0.29	(0.56)			0.64*	(0.30)
						Caecum							
M	0.0			0.68	(1.23)			0.30	(0.53)			1.47	(0.45)
	2.5			0.68	(1.32)			0.30	(0.54)			1.72*	(0.53)
	5.0			0.76*	(1.53)			0.33	(0.62)*			1.82**	(0.57)**
	10.0			0.73*	(1.49)			0.31	(0.58)			2.36**	(0.72)**
F	0.0			0.61	(1.16)			0.27	(0.52)			1.09	(0.53)
	2.5			0.62	(1.26)			0.28	(0.52)			1.23	(0.57)
	5.0			0.67	(1.41)			0.32*	(0.61)*			1.32*	(0.61)
	10.0			0.73	(1.56)**			0.29	(0.58)			1.81**	(0.85)**

Values are means for groups of 12 adult males, 12 F_{3a} females, 23 or 24 other adult females and 17–25 weanlings of either sex. Those marked with asterisks differ significantly from the control value: *$P < 0.05$; **$P < 0.01$.

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