Fd Chem. Toxic. Vol. 20, pp. 371 to 382, 1982
Printed in Great Britain.

0278-6915/82/040371-10$03.00/0

A COMPARISON OF THE EFFECTS OF LACTOSE AND OF TWO CHEMICALLY MODIFIED WAXY MAIZE STARCHES ON MINERAL METABOLISM IN THE RAT

A. HODGKINSON*, D. DAVIS†, J. FOURMAN‡, W. G. ROBERTSON* and F. J. C. ROE§

*MRC Mineral Metabolism Unit, The General Infirmary, Leeds LS1 3EX
†15 Grange Avenue, Spofforth, Harrogate HG3 1AH
‡Department of Anatomy, University of Leeds, Leeds LS2 9JT
§19 Marryat Road, Wimbledon Common, London SW19 5BB

(Received 15 June 1981; revision received 20 January 1982)

Abstract—Diets containing 30% by weight of waxy maize starch, lactose monohydrate, acetylated distarch phosphate (EEC No. 1414) or acetylated distarch adipate (EEC No. 1422) were fed to weanling female Specified Pathogen-Free Sprague–Dawley rats for 1 yr and to similar 9-month-old rats for 34 wk. Behaviour and general health were unaffected by the different diets and there were no diet-related differences in food consumption. At the end of the experiment with 9-month-old rats the mean body weight of the animals receiving lactose was significantly lower than that of the controls receiving starch. The animals receiving the modified starches were slightly but not significantly heavier than the controls at the end of both experiments. The main treatment-related changes in rats on the three test diets were (1) caecal enlargement, (2) increased urinary excretion of calcium, (3) increased renal calcification as measured by chemical analysis of renal tissue obtained at autopsy and (4) increased medullary and pelvic nephrocalcinosis as assessed histopathologically. Acetylated distarch adipate had a slightly greater effect on the above parameters than acetylated distarch phosphate but both modified starches had less effect than lactose. The calcium content of the kidneys, as measured by chemical analysis or histopathology, increased with age, even in the animals receiving the control diet. This change may be due to excessively high concentrations of calcium and phosphorus in all the diets, including the control diet. Cortico-medullary mineral deposits were not a feature in these studies possibly because the diets were not deficient in magnesium. The importance of correct dietary formulation in long-term toxicity studies is emphasized.

INTRODUCTION

There have been several reports that chemically modified starches such as acetylated distarch adipate or hydroxypropyl distarch phosphate cause an enlargement of the caecum and the formation of calcareous deposits within the renal pelvis when introduced into the diet of rats in place of natural starch. Bailey, Cox & Morgareidge (1973), in a 90-day study with weanling Wistar rats, compared the effects of a diet containing up to 25% by weight of hydroxypropyl distarch phosphate with that of a normal diet containing 25% of unmodified starch. Enlarged caeca and calcareous deposits within the renal pelvis and/or pelvic epithelium were reported in 40% of the test animals but not in the control group. Similar findings were reported by de Groot, Til, Feron *et al.* (1974) in a 2-yr study on rats and by Feron, Til & Immel (1978) in an 89-wk study on mice. In addition to enlargement of the caecum and colon and an increased incidence of intratubular nephrocalcinosis, these studies revealed a slight growth retardation, increased urinary excretion of calcium, magnesium and phosphorus and a slightly increased incidence of concrements in the renal pelvic space or bladder. Truhaut, Coquet, Fouillet *et al.* (1979) on the other hand, found that in comparison with unmodified starch there were no significant differences in rate of body growth, serum biochemistry or organ weights in pathogen-free Sprague–Dawley rats given a diet containing 62% of acetylated distarch adipate or acetylated distarch glycerol for 2 yr. Hyperplasia of renal papillary and pelvic epithelia, accompanied by calcified patches in the underlying tissues, were observed but they occurred with approximately equal frequency in both the control and test groups. Some of these discrepancies may be due to differences in the type of starch used but the absence of data relating to organ weights or urine chemistry from the study of Truhaut *et al.* (1979) make it difficult to compare their results with those of previous workers.

It has long been recognized that high dietary intakes of carbohydrates and particularly lactose, increase the absorption of calcium (Bergeim, 1926; Greenwald & Gross, 1929). Other effects include caecal enlargement, increased urinary excretion of calcium, hyperplasia of renal papillary or pelvic epithelia and an increase in the frequency and severity of calcium deposits in the kidney (de Groot & Feron, 1975/6; Feron *et al.* 1978; Vaughan & Filer, 1960).

In the present study we have examined the possibility that chemically modified starches may resemble lactose in their effects on calcium metabolism.

Abbreviations: DS = Degree of substitution; E1414 = Acetylated distarch phosphate; E1422 = Acetylated distarch adipate.

Table 1. *Specifications of test and control starches and of lactose*

Parameter	Control starch	E1414	E1422	Lactose
Ca (ppm)	165	170	260	11
Mg (ppm)	115	70	90	3
P (ppm)	39	154	37	42
Acetyl (% w/w)	—	1·2%*†	2·1%‡	—
Degree of substitution§	—	0·05	0·08	—

*Analysis by Corn Products (Europe) Ltd, Avenue Louise 149, Bte 13, B-1050, Brussels, Belgium.
†Analysis by Scholten-AVEBE, 9607 PN, Foxhol, The Netherlands.
‡Analysis by Laing National (UK) Ltd, Trafford Park, Manchester M17 1BJ.
§The degree of substitution (DS) is the average number of substituents per D-glucose unit of the starch.

EXPERIMENTAL

Test materials. The two chemically modified starches studied were pregelatinized acetylated distarch phosphate (EEC No. 1414) and pregelatinized acetylated distarch adipate (EEC No. 1422). These and the pregelatinized unmodified waxy maize starch were supplied by the Association des Amidonnaires de Mais de la C.E.E., Brussels. Lactose monohydrate (USP XIX/EP 73; 99·8%) was supplied by De Melkindustrie Veghel, Holland. Further details of the specification of the three starches and of the lactose, are given in Table 1.

Animals. Female specified pathogen-free Sprague–Dawley rats, obtained from Charles River, Manston, Kent were used. For Experiment I the animals were aged 3–4 weeks when they were received from the supplier. For Experiment II the animals were ex-breeders, aged 9 months when they were received.

Diets. The diets were prepared in pelleted form, by the Central Institute for Nutrition and Food Research (CIVO), Zeist, Holland. The basic diet used by de Groot *et al.* (1974) formed the basis for all the diets used in the present studies. The control diet comprised 8% fish, 4% meat scraps, 20% soya-bean meal, 7% maize meal, 20% wheat meal, 3% grass meal, 3% brewer's yeast, 0·2% B-vitamins (in mg/kg diet: thiamine HCl, 4; riboflavin, 5; pyridoxine HCl, 2·5; niacin, 2·5; Ca-pantothenate, 15; biotin, 0·1; folic acid, 1·0; vitamin B_{12}, 0·025), 0·4% vitamin A, D_3, E preparation (in IU/g preparation: vitamin A, 2250; vitamin D_3, 750; vitamin E, 25), 0·5% steamed bone meal, 0·4% KH_2PO_4, 0·5% trace mineralized salt (percentage composition: MnO, 2; $ZnCl_2$, 0·05; KI, 0·012; Co acetate.$4H_2O$, 0·04; $FeSO_4.7H_2O$, 2·5; $CuSO_4.5H_2O$, 0·8; NaCl, 94·15) 3% margarine and 30% pregelatinized starch. Molasses was added (50 g/kg diet) as a binding agent for pelleting the diets. The diameter of the pellets was 10 mm. The test diets were prepared in the same way as the control diet and were identical with the control diet except that the 30% content of pregelatinized waxy maize starch was replaced by 30% pregelatinized acetylated di(waxy) starch phosphate (E1414), 30% pregelatinized acetylated distarch adipate (E1422) or 30% lactose and the minerals added to the test diets were adjusted where necessary by adding potassium hydrogen phosphate to raise the concentration of phosphorus, calcium carbonate to raise calcium concentration, and magnesium sulphate to raise magnesium concentration. Fresh batches of food were prepared every 4–8 wk and were stored at −20°C until used. The results of analysis of a typical batch are shown in Table 2.

Experimental design—Experiment I. One hundred weanling rats were allocated at random to four groups of 25 each: group 1, control; group 2, E1414; group 3, E1422; group 4, lactose. The animals were housed in wire-mesh cages in pairs or groups of three and were identified by cage number and tail markings made with coloured felt pens. All animals were weighed, initially at weekly intervals and later at fortnightly intervals. During an acclimatization period of a week all rats were fed on the control diet and were given free access to both food and distilled water.

Subsequently from what was designated day 1 of the experiment, thirteen animals in each group began to receive their respective diets and stayed in their normal cages throughout. One of these from each group was killed at weeks 5, 9, 12, 27, 31 and 39 to check for any progressive or age-related change. Additionally one control animal was killed at week 2 to check autopsy techniques. The remaining twelve animals in each group, divided into four groups of three, spent intermittent weekly stays in separate all-glass metabolism cages (Metabowl, Model 11, Jencons Ltd., Hemel Hempstead, England), which provided reliable measurement of food consumption and good separation of urine and faeces. The timing of these stays and of the switch to their respective diets is shown in Fig. 1. Thus the first three such rats in each group spent week 1 in metabolism cages, started their diet at the beginning of week 2, and subsequently spent weeks 5, 9, 23, 37 and 49 in metabolism cages, with successive groups of three rats following the same pattern but starting one week later. All surviving animals in all groups were killed at week 52.

Experimental design—Experiment II. The design followed essentially that of Experiment I, except that the rats used were approximately 9 months old at the start of the experiment, there were only two interim kills of one animal per group (at weeks 1 and 18) and the four subgroups of three animals from each group for metabolism studies were only observed over three 7-day periods covering weeks 1–4, 15–18 and 27–30. The same observations and measurements were made as in Experiment 1, surviving animals in all groups being killed at week 34. In both experiments, one group 2 metabolism animal died prematurely and a

Table 2. *Results of chemical analysis of blended diets*

Constituent	Control	E1414	E1422	Lactose
Moisture (%)	15·6	16·5	16·4	14·3
Calcium (%)	1·14	1·0	1·2	1·2
Magnesium (%)	0·13	0·12	0·12	0·12
Phosphorus (%)	0·81	0·79	0·78	0·74
Vitamin B_6 (mg/kg)	9·8	7·6	7·7	7·4
Vitamin D (IU/kg)	1330	1550	1250	1690
Oxalate (ppm)	400	450	450	650

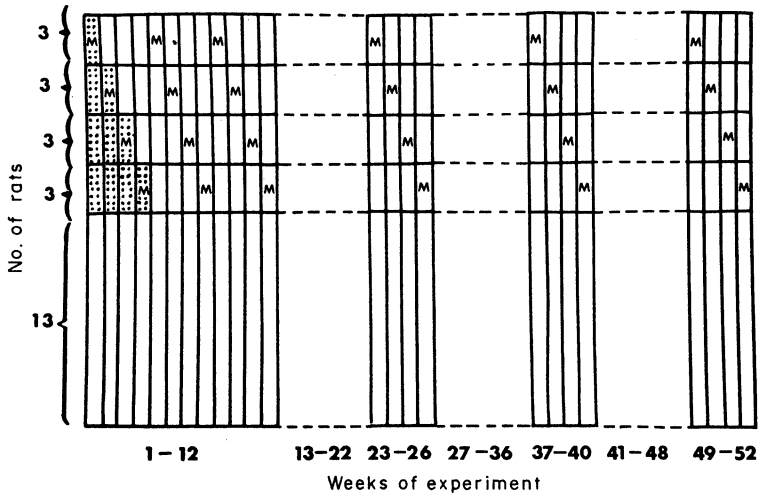

Fig. 1. Design of Experiment I for one group of 25 rats. M denotes a week spent in metabolism cages, the shaded area indicates control diet, and the unshaded area, diet appropriate to the group. The same pattern was followed for each diet group.

non-metabolism animal subsequently took its place in the metabolism cages.

Post mortem *and histopathological examination.* All rats killed, or dying during the experiment, were subjected to a thorough autopsy, using a standardized technique. The bladder and ureter were examined *in situ*, removed, fixed and prepared, with portions of kidney for routine microscopy and histochemistry. One kidney was bisected along its axis and prepared for scanning electron microscopy. At all *post mortem* examinations carried out at the end of the experiment the following organs were weighed: kidneys, lungs, heart, liver, caecum (weight with and without contents), brain, spleen, thymus, adrenals, pituitary and ovaries. These organs, along with samples of uterus, stomach, duodenum, jejunum, ileum, colon, rectum, pancreas, thyroid, parathyroid, mesenteric lymph node and any macroscopically abnormal tissues were preserved in buffered formalin. From 32 of these rats (four from each group) sections of liver, aorta, thyroid, parathyroid, caecum and ovary were stained with H & E and by von Kossa's method for mineralization. Sections of liver and aorta from all animals, abnormal tissue removed at *post mortem* and a total of 52 parathyroid glands, representing each group, were stained with H & E alone. Mineral deposits were localized by the metal substitution method of von Kossa and a number of techniques were used to demonstrate calcium in sections. A scoring system (0–5) was used to assess the amount of silver deposited (calcification) in von Kossa stained sections. Kidney sections from all treatment groups were randomized and scored for deposits localized in cortical and medullary structures. The slides were again randomized and scored for deposits localized in four sites (i) cortex (ii) medulla (iii) pelvic epithelium (iv) urinary space.

Analysis of kidney tissue for minerals. About one half of one kidney, preserved in buffered formalin, was available for analysis from each animal killed at the end of the experiment. It was removed from formalin, dried on tissue paper, transferred to a silica crucible and cut into small pieces with scissors, dried at 100°C to constant weight (48 hr) and then ashed at 550°C overnight. The ash was dissolved in 1 ml of 50% (v/v HCl, then diluted to 10 ml with water. Calcium was determined colorimetrically with cresolphthalein complexone (Morin, 1974) or by atomic absorption spectrometry, good agreement being obtained between the two methods.

Urine analysis. The pH of freshly-passed samples was determined immediately and a microscopic examination was carried out for the presence of crystals, particularly calcium oxalate, calcium phosphate, magnesium ammonium phosphate or uric acid. Oxalate was determined colorimetrically after reduction to glycollic acid with zinc (Hodgkinson & Williams, 1972). Magnesium was determined by atomic absorption spectroscopy, using a lanthanum diluent and phosphate and creatinine by automatic colorimetry. (Technicon Auto Analyzer Methods AA II-04 and AA II-11, respectively).

Statistical analysis. The significance of between-group body weight differences was determined by analysis of covariance using the initial body weight as covariate. Differences in food consumption, urine data, organ weights and calcium content of kidney tissue were investigated by analysis of variance, using logarithmic transformation of the data, if appropriate. For data on rats in the metabolism cages the analyses of variance were carried out separately for each 4-wk sojourn, the data being treated as for a 4 (groups) × 4 (weeks) design. Differences in graded histological scores were tested by the Kruskal–Wallis one-way analysis of variance by ranks (Kruskal & Wallis, 1952; Kruskal & Wallis, 1953) while mammary gland tumour incidences were compared by the method of Peto, Pike, Day *et al.* (1980).

Table 3. *Mortality*

	Control	E1414	E1422	Lactose
		Experiment I		
Initial number of rats	25	25	25	25
Unscheduled deaths (wk)	1(12)	1(9)	0	0
Interim kills	7	6	6	6
Final kills	17	18	19	19
		Experiment II		
Initial number of rats	25	25	25	25
Unscheduled deaths (wk)	2(7,32)	3(3,16,30)	2(29,31)	1(21)
Interim kills	2	2	2	2
Final kills	21	20	21	22

RESULTS

General health and mortality

No animal in either experiment showed any disturbance of behaviour. Two rats in Experiment I and eight in Experiment II died prematurely (Table 3). All deaths were due to subcutaneous tumours in the mammary glands with the exception of three animals, a control animal in Experiment I which died after 12 weeks from wasting due to unknown causes, an animal on E1414 in Experiment I which was found to have a large bladder stone consisting of magnesium ammonium phosphate at 9 wk and a control animal in Experiment II which was found dead after 7 wk from unknown causes.

Body weight

In Experiment I the mean body weight of the animals receiving lactose did not differ significantly from that of the controls but in Experiment II the mean body weight of animals on the lactose diet was significantly lower ($P < 0.01$). The mean weights of the animals receiving acetylated distarch phosphate (E1414) and acetylated distarch adipate (E1422), on the other hand, tended to be slightly higher than those of the controls, though the difference did not reach the 5% level of significance in either experiment (Fig. 2).

Food consumption

The mean daily weight of food consumed by animals while they were in the metabolism cages decreased progressively with increasing age of the animals, from about 18 g per day in weanling rats weighing 50–100 g to about 2 g per day in 18-month-old rats weighing 400–500 g. Food consumption data were not available from animals that were not in metabolism cages, for comparison, but the very low amount of food consumed towards the end of the study is considered to be artefactual and secondary to stress associated with residence in all-glass metabolism cages. No statistically significant differences between groups were seen except that in Experiment I the lactose group ate about 2 g/rat/day more than did the controls from weeks 5 to 12.

Urine analysis

The results from two of the six 4-wk periods in metabolism cages in Experiment I and for two of the three 4-wk periods in Experiment II, each a mean from 12 animals per group, are summarized in Table 4. These findings and those at the other time points (not presented for reasons of space) are summarized below.

Volume. The mean daily urine volume was appreciably higher in the animals receiving lactose than in those receiving the control diet although this was only significant in Experiment I. No significant differences were seen in animals receiving E1414 and E1422.

pH. The mean pH of freshly voided urine was reduced in rats receiving lactose compared with the control animals, this being highly significant ($P < 0.001$) in Experiment I. No significant change was observed in the animals receiving the modified starches.

Calcium. Both the mean concentration of calcium in the urine and the total daily excretion of calcium were significantly increased in the lactose group. This increase was seen at all times except weeks 1–4 in both experiments and was often very highly significant ($P < 0.001$). Increases in both calcium measures were seen at nearly all time points for both the modified starches. This increase was less marked in the E1414 group, where it was never statistically significant, and more marked in the E1422 group where a

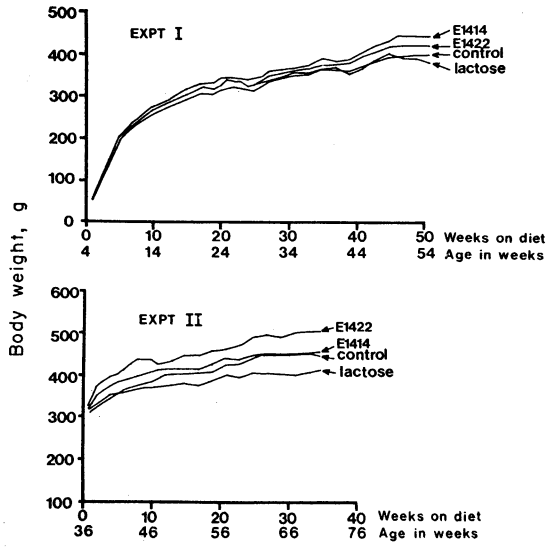

Fig. 2. Changes in the mean body weights with age and time on diet.

Table 4. *Urine composition*

	Experiment I				Experiment II					
	Weeks on diet	Control	E1414	E1422	Lactose	Weeks on diet	Control	E1414	E1422	Lactose
Volume (ml/day)	23–26	18·7	21·7	18·8	32·1*	15–18	11·2	17·5	12·7	18·0
	49–52	18·8	17·3	15·4	27·9	27–30	11·7	15·8	12·9	16·1
pH	23–26	NA	NA	NA	NA	15–18	6·76	6·45	6·71	6·24†
	49–52	9·13	7·22	7·01	6·13†††	27–30	6·93	6·76	7·04	6·55
Calcium (mmol/litre)	23–26	2·03	2·73	4·97***	6·96***	15–18	2·60	2·88	3·59	6·01***
	49–52	3·57	4·00	5·13	6·90**	27–30	3·10	2·96	4·61	6·71**
Calcium (μmol/day)	23–26	41·3	53·2	83·7***	177·8***	15–18	23·3	48·9	46·3	115·2***
	49–52	58·3	64·9	61·2	154·5***	27–30	30·2	45·0	56·0	92·7***
Phosphate (mmol/litre)	23–26	34·8	34·0	38·0	31·5	15–18	51·0	39·0	51·1	46·1
	49–52	33·5	37·4	44·3	30·0	27–30	46·5	44·2	55·3	42·6
Magnesium (mmol/litre)	23–26	7·45	8·58	12·57**	8·84	15–18	6·61	6·04	7·58	7·98
	49–52	6·48	7·13	7·78	6·73	27–30	7·67	8·09	7·80	7·86
Oxalate (mmol/litre)	23–26	2·61	3·19	2·13	1·63	15–18	1·84	1·17†	1·63	1·29
	49–52	1·73	1·25†	1·33†	1·04††	27–30	1·59	1·46	1·67	1·23
Creatinine (mmol/litre)	23–26	6·67	6·45	5·95	4·83†	15–18	11·2	7·78	10·9	7·73
	49–52	6·13	6·95	7·00	4·07††	27–30	9·53	8·02	9·70	6·82
Calcium phosphate product (mmol/litre)^2	23–26	71	96	208**	245**	15–18	151	130	189	267*
	49–52	129	153	253	243	27–30	165	137	257	312
Calcium oxalate product (mmol/litre)^2	23–26	4·23	6·60	11·5*	10·9*	15–18	4·94	3·38	5·84	7·17
	49–52	6·83	5·39	7·58	7·97	27–30	5·36	4·80	7·96	9·02

NA = Data not available

Values marked with asterisks show a significant increase and those marked with daggers a significant decrease (analysis of variance) compared with the control (* or † $P < 0·05$; ** or †† $P < 0·01$; *** or ††† $P < 0·001$).

number of tests showed a significant or near significant difference.

Phosphate, oxalate, magnesium and creatinine excretion. There were only minor differences in the urinary concentrations of phosphate, oxalate, magnesium and creatinine between rats on the four diets. The oxalate and creatinine concentrations actually decreased on the lactose diet, as a consequence of the increased urine volumes. There was an increase in urinary magnesium concentration in rats fed on E1422 in both experiments but this was only significant in two cases (weeks 9–12 and 23–26 in Experiment I).

Calcium × phosphate concentration product. This measure of the tendency for calcium phosphate to precipitate from the urine was significantly raised on the lactose diet and to a lesser extent on the E1422 diet, whereas the product was not significantly changed on E1414.

Calcium × oxalate concentration product. The calcium × oxalate product tended to be higher on the lactose and E1422 diets though the differences were generally not statistically significant.

Occurrence of calcium phosphate and calcium oxalate crystals in urinary sediment. Crystals of calcium phosphate were observed in over half the samples of fresh urine collected during weeks 49–52 in Experiment I or during weeks 27–30 in Experiment II but there was no treatment-related pattern, the number of rats (out of 12) in which crystals were observed being in Experiment I: control 6, E1414 7, E1422 7 and lactose 5 and in Experiment II: control 8, E1414 5, E1422 9 and lactose 4. Crystals were also seen in a third of the samples seen during weeks 37–40 in Experiment I or weeks 15–18 in Experiment II but again there was no significant treatment effect. Calcium oxalate crystals were observed in only one (control) sample in Experiment I and in two samples (one control and one E1422) in Experiment II. Crystals of magnesium ammonium phosphate were not encountered in either of the experiments.

Analysis of faeces for minerals

No pronounced differences in faecal concentration of calcium, magnesium or phosphorus were observed between groups. Most of the differences that occurred reflected differences in food intake; when these were allowed for there was an apparent slight deficit in the faecal concentration of calcium in rats on the lactose diet, compared with the controls, but no detectable deficit for those on the E1414 or E1422 diets.

Calcium content of kidney tissue

There were considerable variations between individual animals but calcium concentrations were nearly all higher in the lactose-treated animals compared with the controls in Experiment I, the mean concentration (22·9 μmol/g dry weight of tissue) being 2·4 times higher than that of the controls and the difference was highly significant ($P < 0.001$). The mean concentrations in the E1414 and E1422-treated animals were also significantly ($P < 0.01$) higher than that of the controls, though the increase was not as pronounced as in the case of lactose (Table 5). The results for the older rats of Experiment II followed a similar pattern to that for Experiment I except that values, including those in the controls, tended to be higher than in the younger animals and a significant excess was seen only in the lactose group ($P < 0.05$).

Organ weights

All three treated groups in both experiments exhibited caecal enlargement which was most marked and highly significant for the lactose group (Table 6). The lactose group also had raised relative liver weights, raised relative and absolute adrenal weights, reduced relative and absolute thymus weights, raised relative kidney weights and raised relative and absolute spleen weights in both experiments though the increases for the kidney and spleen were not significant in Experiment I. The E1422 group showed a significant increase in absolute weight of a number of organs (kidneys, liver, heart, spleen) in Experiment II but in this experiment the total rat weight was also increased in this group. When relative weights were considered, where significant differences existed between the E1414 or E1422 groups and the controls, these did not seem indicative of toxicological effect since there was no consistent pattern for the two experiments and the weight changes were not associated with any detectable histopathological changes.

Histological findings in the kidney

Calcium deposits were observed in four different sites:

(i) in the cortex, deposited in the basement membrane surrounding the tubules or in the interstitial space,
(ii) in the medulla, as intratubular and extratubular deposits,
(iii) within the cells of the normal or hyperplastic epithelium lining the pelvic urinary space or deposited beneath it, or,
(iv) in some sections, as casts and amorphous deposits within the pelvic urinary space.

The cortico-medullary pattern of calcification typical of hyper-vitaminosis D or of magnesium deficiency was not encountered in either experiment. The degree of calcification based upon a subjective system of scoring for rats killed at termination is summarized in

Table 5. *Calcium content of kidney tissue from chemical analysis*

	Kidney calcium level (μmol/g dry wt)*			
	Control	E1414	E1422	Lactose
Experiment I	9·4 ± 0·5 (17)	14·7 ± 1·9 (18)	13·2 ± 1·3 (19)	22·9 ± 2·8 (19)
Experiment II	25·3 ± 3·9 (21)	34·7 ± 7·4 (20)	33·7 ± 4 (21)	59·3 ± 10·6 (22)

*Values are means ± SEM for the numbers of animals given in parentheses.

Table 6. *Absolute and relative organ weights*

	Absolute organ weights (g)				Relative organ weights (g/100 g body wt)			
	Control	E1414	E1422	Lactose	Control	E1414	E1422	Lactose
				Experiment I				
Caecum and contents	4·98	6·28***	6·27**	10·16***	1·20	1·38*	1·51*	2·54***
Caecum empty	1·49	1·67*	1·77**	2·44***	0·36	0·37	0·42*	0·61***
Kidneys	3·46	3·44	3·43	3·68	0·84	0·76	0·80	0·92
Liver	15·15	16·16	15·95	15·96	3·67	3·54	3·66	3·96
Heart	1·32	1·33	1·34	1·30	0·32	0·29	0·31	0·33
Brain	1·95	1·92	1·92	1·93	0·48	0·42††	0·45	0·49
Adrenal	0·08	0·08	0·09	0·14††	0·021	0·018	0·022	0·035†
Spleen	0·58	0·57	0·62	0·65	0·14	0·13	0·14	0·16
Thymus	0·42	0·42	0·42	0·26††	0·10	0·09	0·09	0·06†††
				Experiment II				
Caecum and contents	6·30	7·39**	9·18***	10·71***	1·40	1·61	1·77**	2·56***
Caecum empty	1·86	2·09*	2·59***	2·73***	0·41	0·45	0·49*	0·66***
Kidneys	3·16	3·43*	3·50**	3·34	0·69	0·74	0·67	0·80**
Liver	15·11	16·55	18·07***	15·98	3·29	3·54*	3·42	3·82***
Heart	1·32	1·44*	1·50***	1·32	0·29	0·31	0·29	0·32
Brain	2·02	1·91†	1·97	1·98	0·45	0·42	0·38†	0·48
Adrenal	0·09	0·10	0·09	0·10*	0·020	0·021	0·017	0·024***
Spleen	0·53	0·59*	0·69***	0·70***	0·12	0·13	0·13	0·17***
Thymus	0·44	0·36	0·62	0·28††	0·09	0·07	0·11	0·07††

Values marked with asterisks show a significant increase and those marked with daggers a significant decrease (analysis of variance) compared with the control (* or † $P < 0·05$; ** or †† $P < 0·01$; *** or ††† $P < 0·001$).

Table 7. Compared with rats in the control, E1414 and E1422 groups mineral deposits of all the kinds listed above were most prominent in the lactose group, with significant excesses in Experiment I for the pelvic epithelium ($P < 0·01$) and in Experiment II for the cortex ($P < 0·001$), pelvic epithelium ($P < 0·001$) and urinary space ($P < 0·05$). There was also a general tendency for rats in the E1414 and E1422 groups to show greater deposition than those in the control groups but this was less marked than for lactose and only statistically significant ($P < 0·05$) in the case of the urinary space for the E1422 group in Experiment II. Calcification associated with the pelvic epithelium and urinary space, as illustrated in Figs 3 and 4, represents a change that has, in recent years, been referred to as pelvic nephrocalcinosis (PN). Thus, all three treatments were associated with PN but lactose had more effect than either of the two modified starches.

Histopathological findings in other tissues

All organs and tissues exhibiting macroscopic changes and in addition the samples taken of bladder, ureters, liver, aorta, parathyroids, caecum and ovaries were examined microscopically. No mineral deposits were found in the bladders or ureters.

Table 7. *Calcification of the kidney in rats killed at termination determined by histological assessment*

Diet	No. of rats	Mean calcification score* in the Cortex	Medulla	Pelvic epithelium	Urinary space
		Experiment I			
Control	17	0·18	0·06	0·65	0·06
E1414	18	0·28	0·22	1·28	0·94
E1422	19	0·26	0·26	1·42	0·63
Lactose	19	0·37	0·26	2·59	0·95
		Experiment II			
Control	21	1·48	0·33	0·38	0·24
E1414	20	1·15	0·40	0·85	0·45
E1422	21	0·91	0·95	1·05	0·67
Lactose	22	2·68	1·05	2·46	1·91

*The calcification scores were attributed as follows: 1 = trace, 2 = small deposits, 3 = small generalized deposits, 4 = larger generalized deposits, 5 = large deposits.

Table 8. *Incidence of fatty change in the liver of rats killed at termination*

Group	No. of animals examined	Incidence of fatty change: No change	1 Minimal	2 Slight	3 Moderate	4 Severe	Any change (%)	Mean score
			Experiment I					
Control	16	14	2	0	0	0	12·5	0·13
E1414	18	12	1	4	1	0	33·3	0·67
E1422	15	11	2	2	0	0	26·7	0·40
Lactose	19	19	0	0	0	0	0·0	0·00
			Experiment II					
Control	21	14	4	2	1	0	33·3	0·52
E1414	20	15	4	1	0	0	25·0	0·30
E1422	21	12	1	5	3	0	42·9	0·95
Lactose	21	21	0	0	0	0	0·0	0·00

The incidence of fatty change in the liver is summarized in Table 8. Although there was no significant difference between incidence in the control, E1414 and E1422 groups, this incidence was significantly higher than the zero incidence seen in the lactose group in both experiments (Experiment I, $P < 0.05$; Experiment II, ($P < 0.01$).

Aortic calcification was not seen in Experiment I but was seen in Experiment II in all groups. No difference was seen between the mean scores in the control, E1414 and E1422 groups, but there was a highly significant increase ($P < 0.001$) in the lactose group (Table 9).

Focal hyperplasia of the parathyroid was seen in two rats in Experiment II, one a control and one a lactose animal and fibrosis was seen in the parathyroid of one rat of the E1414 group in Experiment II. There was therefore no treatment-related excess of hypertrophy or neoplasia to account for the excessive calcification seen in lactose-treated rats and to a lesser extent in rats exposed to E1414 or E1422.

No histological abnormality was seen in samples of caeca (four from each group in each experiment) despite the macroscopically visible enlargement. Inflammation of the caecum in one E1422 rat of Experiment II was not considered to be related to treatment. Similarly, enlargement and necrosis of the ovary of a lactose-fed rat in Experiment I and various other lesions seen in tissues exhibiting macroscopic changes (Experiment I, E1422, endometrial polyp; Experiment II, E1414, cystic degeneration of adrenal; E1422, pituitary adenomas in two rats) were thought to be attributable to background pathology.

Mammary gland tumours

Mammary gland tumours were observed in 23 animals (Table 10). All except four tumours were examined histologically and all were found to be fibroadenomas. Although the incidence of these tumours was slightly lower in the controls than in other groups, there was no statistically significant between-group difference, using Peto's method of analysis ($\chi^2 = 4.6$ on 3 d.f., $P < 0.1$). This type of tumour is a common feature of *ad lib.*-fed laboratory rats (Roe, 1979).

DISCUSSION

The two modified starches we have examined appear to have similar, but less marked effects to those of lactose in causing caecal enlargement and in increasing absorption and excretion of calcium. Caecal enlargement is probably due to an increased amount of osmotically-active material in the intestine which, in turn, may be caused by a failure to digest, or absorb completely the increased quantity of dietary carbohydrate before the food bolus reaches the caecum (Leegwater, de Groot & van Kalmthout-Kuyper, 1974; Walker, 1978). The hypercalciuria which occurs after the ingestion of lactose is secondary to increased intestinal absorption of calcium, though the precise mechanism is still not clear (Norman, Morawski & Fordtran, 1980; Pansu, Bellaton & Bronner, 1979).

Various types of renal calcification (nephrocalcinosis) have been described in the rat. These include (1) cortical calcification, seen after giving ip injections of calcium salts sufficient to cause hypercalcaemia and

Table 9. *Incidence of aortic calcification in Experiment II in rats killed at termination*

Group	No. of animals examined	Incidence of aortic calcification: No change	1 Minimal	2 Slight	3 Moderate	4 Severe	Any change (%)	Mean score
Control	18	8	2	4	4	0	55·6	1·22
E1414	19	7	3	2	5	2	63·2	1·58
E1422	21	10	2	5	2	2	52·4	1·24
Lactose	19	3	0	5	3	8	84·2	2·68

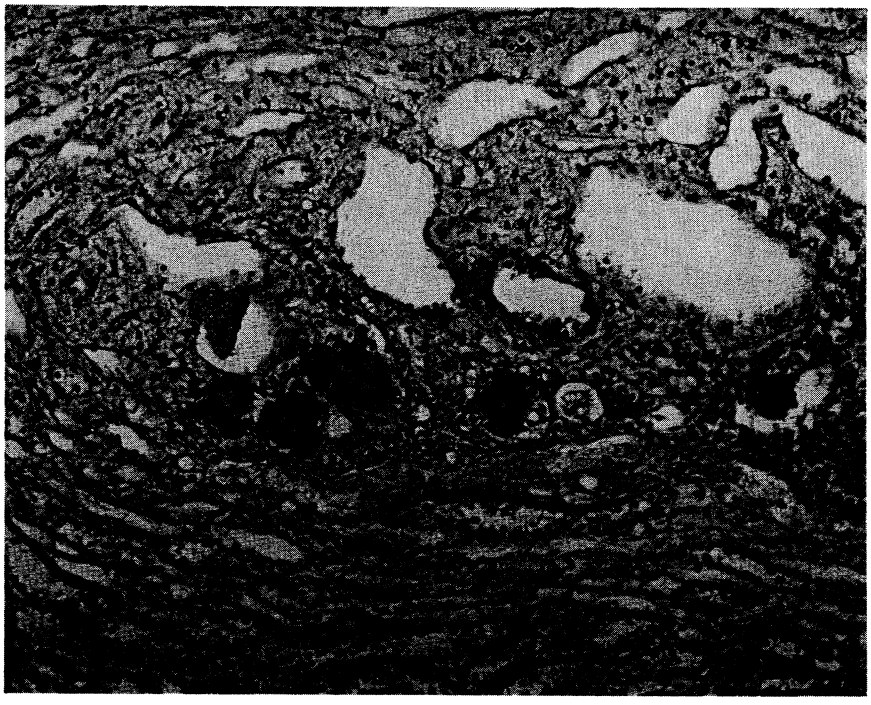

Fig. 3. Pelvic nephrocalcinosis. Epithelial hyperplasia, adhesions and mineral deposits in a recess of the pelvic fornix from a 75-wk-old control rat. Experiment II, H & E × 80.

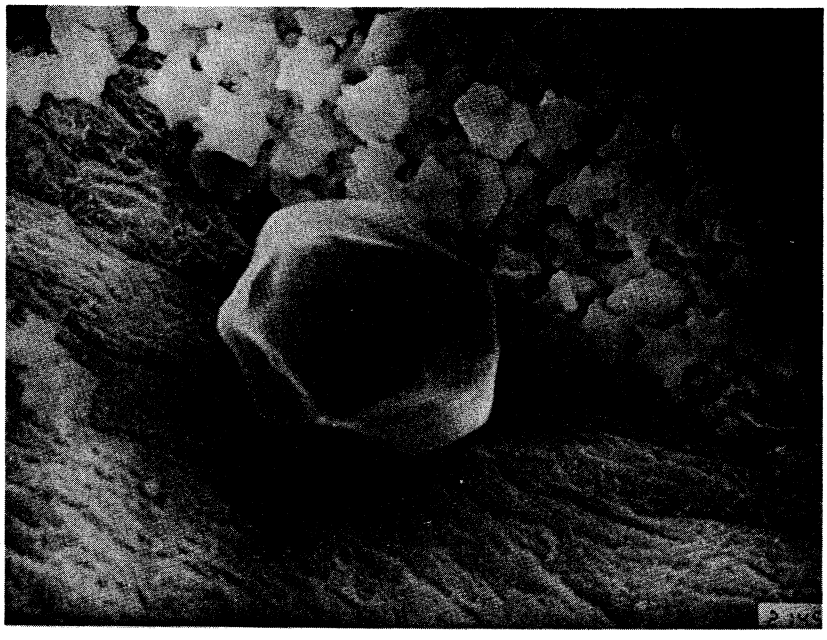

Fig. 4. Crenated red blood cells and a mineral deposit lying on epithelium lining the urinary space of the pelvic fornix. S.E.M. × 3000.

Table 10. *Incidence of mammary gland tumours*

	Incidence of mammary tumours			
Diet...	Control	E1414	E1422	Lactose
Experiment I				
Unscheduled deaths	0/1	0/1	0/0	0/0
Interim	1/7	0/6	1/6	2/6
Final kills	0/17	1/18	3/19	1/19
Total	1/25	1/25	4/25	3/25
Experiment II				
Unscheduled deaths	1/2	3/3	2/2	1/1
Interim kills	0/2	0/2	0/2	0/2
Final kills	0/21	3/20	2/21	2/22
Total	1/25	6/25	4/25	3/25
Experiments I & II combined				
Observed tumours	2	7	8	6
Expected tumours	5·7	5·6	5·8	5·9

hypercalciuria (Fourman, 1959) (2) cortico-medullary calcification, such as occurs after administering parathyroid hormone, vitamin D or inorganic phosphate (Fourman, 1959; Holdsworth & Hodgkinson, 1961) or under conditions of dietary magnesium deficiency (Du Bruyn, 1972; Schneeberger & Morrison, 1965); (3) a more recently recognized calcification in the pelvic region, particularly in the calyx, usually accompanied by calcified deposits in the pelvic lumen (pelvic nephrocalcinosis) (Casey, Ayers & Robinson, 1978). All three types of calcification have been observed in rats fed high lactose diets but the pelvic type is generally predominant (de Groot & Feron, 1975/6; Feron *et al.* 1978; Sambhavaphol, Bosworth & McCay, 1958).

The two modified starches had similar, but less marked, effects to those of lactose, the effects generally decreasing in the order: lactose > E1422 > E1414. There was no evidence that lactose or either of the modified starches had any effect on any parameter other than caecal weight and mineral metabolism.

The extent of the effect of chemically-modified starches on mineral metabolism appears to be related to the type of chemical substitution, the dose-level and possibly the degree of substitution of the starch (Chen, Tsai & Nesheim, 1980). The more substituted starch used in this study (E1422; DS = 0·08) had, in the case of most of the measured parameters, more effect on mineral metabolism than the less substituted preparation (E1414; DS = 0·05), but this difference could be due to differing effects of phosphate and adipate substitution rather than to variations in the degree of substitution.

The data summarized in Tables 5 and 7 show that the calcium content of the kidneys was higher in older rats (Experiment II) than in younger rats on the same diet (Experiment I) even for rats on the control diet. This increase could be due to an excessive intake of calcium or phosphate since the concentrations in the diet were both about twice those currently recommended for optimum growth, gestation or lactation by the National Academy of Sciences (1978). These high intakes were chosen deliberately to make the present results comparable with those of previous workers but further information is clearly required regarding the optimum concentrations of calcium and phosphorus for use in long-term toxicity studies. The calcium content of the kidney might well be a useful index for monitoring studies of this kind.

Acknowledgements—The authors wish to thank Mr P. N. Lee for statistical advice, Dr W. F. Vogel and Mr E. G. Rapp for helpful comments and Mr Neil Crossley for expert technical assistance.

REFERENCES

Bailey, D. E., Cox, G. G. & Morgareidge, K. (1973). Subacute Feeding Studies in Rats with Hydroxypropyl Distarch Phosphate. Report No. 1420. Food and Drug Research Laboratories Inc., New York.

Bergeim, O. (1926). Intestinal chemistry. V. Carbohydrates and calcium and phosphorus absorption. *J. biol. Chem.* **70,** 35.

Casey, H. W., Ayers, K. M. & Robinson, F. R. (1978). Urolithiasis. In *Pathology of Laboratory Animals.* Edited by K. Benirschke, F. M. Garner & T. C. Jones, Vol. 1, p. 157. Springer-Verlag, Berlin.

Chen, S. C.-H., Tsai, S. & Nesheim, M. C. (1980). Response of rats fed diets low in glucose and glucose precursors to low levels of glucose, starch and chemically modified starch. *J. Nutr.* **110,** 1023.

de Groot, A. P. & Feron, V. J. (1975/1976). Effects of Very High Dietary Levels of Lactose on the Kidneys of Rats. Report No. 4812, Central Institute for Nutrition and Food Research (CIVO/TNO), Zeist, The Netherlands.

de Groot, A. P., Til, H. P., Feron, V. J., Dreef-van der Meulen, H. C. & Willems, M. I. (1974). Two-year feeding and multigeneration studies in rats on five chemically modified starches. *Fd Cosmet. Toxicol.* **12,** 651.

Du Bruyn, D. B. (1972). Nephrocalcinosis in the white rat. Part II. The relationship between dietary magnesium, calcium and phosphorus content and kidney calcification and bone magnesium. *S. Afr. med. J.* **46,** 1588.

Feron, V. J., Til, H. P. & Immel, H. R. (1978). Chronic (89-week) Feeding Study with Hydroxypropyl Distarch Phosphate, Starch Acetate, Lactose and Sodium Alginate in Mice. Report No. 5690. Central Institute for Nutrition and Food Research (CIVO/TNO), Zeist, The Netherlands.

Fourman, J. (1959). Two distinct forms of experimental nephrocalcinosis in the rat. *Br. J. exp. Path.* **40,** 464.

Greenwald, I. & Gross, H. (1929). The prevention of the tetany of parathyroidectomized dogs. II. Lactose-containing diets. *J. biol. Chem.* **32,** 531.

Hodgkinson, A. & Williams, A. (1972). An improved colorimetric procedure for urine oxalate. *Clinica chim. Acta* **36,** 127.

Holdsworth, M. J. & Hodgkinson, A. (1961). The hexosamine content of the kidney in experimental renal calcification. *Br. J. exp. Path.* **42,** 331.

Kruskal, W. H. & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *J. Am. statist. Ass.* **47,** 583.

Kruskal, W. H. & Wallis, W. A. (1953). Use of ranks in one-criterion variance analysis. *J. Am. statist. Ass.* **48,** 907.

Leegwater, D. C., de Groot, A. P. & van-Kalmthout-Kuyper, M. (1974). The aetiology of caecal enlargement in the rat. *Fd Cosmet. Toxicol.* **12,** 687.

Morin, L. G. (1974). Direct colorimetric determination of serum calcium with O-cresolphthalein complexone. *Am. J. clin. Path.* **61,** 114.

National Academy of Sciences (1978). *Nutrient Requirements of Laboratory Animals 10.* 3rd. Ed., p. 7. NAS/NRC, Washington, DC.

Norman, D. A., Morawski, S. G. & Fordtran, J. S. (1980). Influence of glucose, fructose and water movement on calcium absorption in the jejunum. *Gastroenterology* **78,** 22.

Pansu, D., Bellaton, C. & Bronner, F. (1979). Effect of lactose on duodenal calcium-binding protein and calcium absorption. *J. Nutr.* **109,** 508.

Peto, R., Pike, M. C., Day, N. E., Gray, R. G., Lee, P. N., Parish, S. Peto, J., Richards, S. & Wahrendorf, J. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In *Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal.* p. 311. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Suppl. 2. International Agency for Research on Cancer, Lyon.

Roe, F. J. C. (1979). Aetiology of breast cancer: a brief review. *Investve Cell Path.* **2,** 45.

Sambhavaphol, P., Bosworth, E. B. & McCay, C. M. (1958). Calculi and kidney calcification from feeding milk diets to rats and hamsters. *Am. J. clin. Nutr.* **6,** 159.

Schneeberger, E. E. & Morrison, A. B. (1965). The neuropathy of experimental magnesium deficiency. *Lab. Invest.* **14,** 674.

Truhaut, R., Coquet, B., Fouillet, X., Galland, D., Guyot, D., Long, D. & Rouaud, J. L. (1979). Two-year oral toxicity and multigeneration studies in rats on two chemically modified maize starches. *Fd Cosmet. Toxicol.* **17,** 11.

Vaughan, O. W. & Filer, L. J. (1960). The enhancing action of certain carbohydrates on the intestinal absorption of calcium in the rat. *J. Nutr.* **71,** 10.

Walker, R. (1978). Some observations on the phenomenon of caecal enlargement in the rat. *Chem. Toxicol. Fd* **3,** 339.