The effect of trypsin inhibitor on the pancreas and small intestine of mice

BY Y. C. GE

Research School of Biological Science, The Australian National University, Canberra, Australian Capital Territory 2601, Australia

AND R. G. H. MORGAN

Department of Physiology, University of Western Australia, Nedlands, Western Australia 6009, Australia

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Pancreatic and intestinal growth rates were measured in mice fed on raw soya-bean flour (RSF) for up to 24 weeks. Control animals were fed on standard chow. The effects of RSF on the mouse pancreas resembled that seen in rats, showing hypertrophy with some hyperplasia. A marked increase in small intestinal weight was also found in mice fed on RSF but not in rats fed on this diet. Histological studies showed an increase in both villous and crypt thicknesses in the small intestine from these mice, and DNA, RNA and protein measurements indicated that the increase in intestinal weight was due to hypertrophy and hyperplasia of the mucosal layer. To determine whether the intestinal growth in mice fed on RSF was purely a response to the trypsin inhibitor (TI) component of the diet, pancreatic and intestinal growth rates were also determined in mice fed on the synthetic trypsin inhibitor camostate, at levels of 0·5 or 2 g/kg in rat chow, for periods of 1–8 weeks. Control animals were fed on standard chow. RSF and 0·5 g camostate/kg had similar trypsin inhibitor activities (measured against bovine trypsin), and both caused similar increases in pancreatic weight, DNA, RNA and protein content. However, 0·5 g camostate/kg did not affect small intestinal weight. Chow containing 2 g camostate/kg contained twice as much TI activity as the RSF diet but produced only a small increase in small intestinal weight at 2 and 8 weeks. This intestinal growth was significantly less than that seen with RSF. The present study shows that, in the mouse, RSF or a diet containing camostate in the appropriate dose produces pancreatic growth comparable to that seen in the rat. RSF also causes intestinal growth, but camostate-containing diets have little or no effect on the growth of the intestine.

Trypsin inhibitors: Pancreas: Small intestine: Mice

A number of studies have investigated the effect of feeding raw soya-bean flour (RSF) on the growth of the pancreas in the rat (Rackis *et al.* 1963; Nitsan & Bondi, 1965; Beswick *et al.* 1971; Fölsch *et al.* 1974; Melmed *et al.* 1976; Crass & Morgan, 1982; Naim *et al.* 1982; Oates & Morgan, 1982), but fewer studies have looked at the effect of this diet in the mouse (Niederau *et al.* 1987; Tudor & Dayan, 1987; Gumbmann *et al.* 1989). In the rat, feeding RSF rapidly leads to both hypertrophy and hyperplasia of the pancreas, while prolonged feeding leads to the development of numerous neoplastic foci (atypical acinar cell nodules), frequently progressing to invasive carcinoma (McGuinness *et al.*, 1980, 1982). In this species RSF also potentiates pancreatic carcinogens such as azaserine (Morgan *et al.* 1977; Levison *et al.* 1979; McGuinness *et al.* 1983; Roebuck *et al.* 1987).

RSF contains a number of biologically active compounds which have been implicated in the effects of this diet on the pancreas, but almost certainly the most important of these are

trypsin inhibitors (TI). These components are active against many mammalian trypsins (Struthers & MacDonald, 1983; Struthers *et al.* 1983). It has been proposed that when luminal levels of trypsin are reduced the release of the gut hormone cholecystokinin (CCK) is stimulated, either because trypsin normally suppresses CCK release (Green & Lyman, 1972) or because CCK release is stimulated by a peptide which is normally rapidly destroyed by trypsin. This peptide may be secreted by the pancreas ('monitor peptide', Iwai *et al.* 1988; Fushiki & Iwai, 1989) or the gut itself (Guan *et al.* 1990).

Feeding RSF also causes pancreatic enlargement in the mouse, but even prolonged exposure does not significantly increase the incidence of pancreatic neoplasms (Hasdai & Liener, 1986; Gumbmann *et al.* 1989). As part of a study to identify the reasons for this species difference we have studied the effect of feeding RSF for up to 24 weeks in the mouse. The results confirmed that pancreatic enlargement occurs, largely due to hypertrophy, but an unexpected finding was that this diet also causes marked intestinal growth in the mouse.

CCK is a potent stimulant of pancreatic secretion, and prolonged exposure to this hormone results in pancreatic hypertrophy. It is likely that much of the growth-stimulating effect of CCK on the pancreas is due to its specific secretory effects on the pancreatic acinar cells. In support of this supposition, Lütcke *et al.* (1987) found that when pancreatic secretion was stimulated in the rat by prolonged infusion of caerulein, some animals showed a fall in pancreatic amylase (*EC* 3.2.1.1) level while others did not ('non-responders'). Marked increases in the incorporation of [^{3}H]thymidine into DNA were seen only in animals in which significant depletion of amylase occurred. However, CCK has no recognized secretory effects on the small intestine so any stimulation of growth of the intestine as part of a 'work hypertrophy' is unlikely. Furthermore, RSF or CCK do not affect small intestinal growth in the rat. Because of the possibility that the intestinal growth seen in the mouse was due to a component of RSF other than trypsin inhibitor the effect of a pure synthetic trypsin inhibitor, camostate mesylate, on pancreatic and intestinal growth in the mouse was also studied.

MATERIALS AND METHODS

Animals

Male mice of the locally outbred Swiss strain Arc(s) were obtained from the Animal Resources Centre, Willetton, Western Australia. These animals were 28 d old (mean weight 20·6 g) at the start of the experiment. In Expt 3 outbred Wistar-derived rats from the Animal Resources Centre were fed on RSF or chow from 6 weeks of age (about 200 g).

Diets

Food and water were available *ad lib.* RSF and heated soya-bean flour (HSF) was obtained from Soy Products of Australia Pty. Ltd, Bayswater, Victoria, and supplemented with vitamins and minerals as recommended by Fölsch & Wormsley (1974). Soya-bean flour was fed in a pelleted form since mice fed on powdered diets survive only a few weeks, dying of severe respiratory infection, probably because they cannot be effectively excluded from feeding trays and inhale the soya-bean flour dust. In early studies cubed RSF was obtained from Special Diet Services Ltd, Witham, Essex, UK, but in most studies soya-bean flour pellets (RSF and HSF) were made as described by Ge & Morgan (1990). There was no difference in the response to these RSF diets. The trypsin inhibitor content of the RSF diets varied between 45 and 56 mg bovine trypsin inhibited/g sample, measured by the method of Hamerstrand *et al.* (1981).

Camostate (Ono Pharmaceutical Company, Osaka, Japan) diets were made by combining either 0·5 or 2 g camostate/kg with powdered mouse stock cubes and pelleting

the mixture as described previously. The method for measuring trypsin inhibitor content of these diets was modified to extract the diets in water (about pH 6) rather than the recommended dilute NaOH, since at alkaline pH most of the camostate activity was lost. The 0·5 g camostate/kg diet inhibited 27–30 mg and the 2 g camostate/kg diet 85–90 mg bovine trypsin/g diet.

RSF and HSF contained about 200 g fat, 400 g protein and 250 g carbohydrate/kg, while mouse stock cubes (control and camostate diets) contained 50 g fat, 220 g protein and 520 g carbohydrate/kg. Pelleted HSF inhibited about 5 mg trypsin/g diet while stock cubes inhibited < 2 mg trypsin/g diet.

Experimental groups

Expt 1. Mice were 28 d old at the start of the experiment. Forty-eight animals in each group were fed on RSF or chow. Eight animals from each dietary group were killed after 1, 2, 4, 8, 12 and 24 weeks on these diets. All animals were fasted overnight and had free access to water before being killed.

Animals were weighed before killing, then anaesthetized with diethyl ether and killed by decapitation. For all animals in each group the pancreas was removed, rapidly trimmed of fat and lymph tissue and weighed, and the small intestine from the pylorus to the end of the ileum was removed and weighed. In four of these animals the pancreas was frozen on dry ice for measurement of DNA, RNA and protein later on the same day.

In four mice fed on RSF or chow for 8 weeks a segment of duodenum taken about 50 mm from the pylorus was washed clear of food residue, opened, and fixed flat in buffered formol saline (9 g NaCl/l). The fixed tissue was embedded in paraffin and sectioned along the line of the gut for histological study.

A separate group of mice were fed on chow, HSF or RSF. Five animals from each dietary group were killed after 4 weeks on the diets. The gut was removed and a 50 mm segment taken from the upper end of the duodenum. The segment was washed with saline, blotted dry and weighed, then opened and the mucosa scraped off with a glass slide. The mucosa and remaining muscle layer were weighed separately, following which the tissue was assayed for DNA, RNA and protein contents.

Expt 2. Three groups, each of four mice, 28 d old at the start of the experiment, were fed on RSF, HSF and chow for 3 weeks. The animals were then killed and the pancreas, small intestine, caecum and colon removed as described previously. All gut segments were weighed, then the contents of the intestinal segments washed out by syringing with 20–30 ml of ice-cold saline. The intestinal segments were suspended vertically for 15 s for drainage, before weighing again. The caeca were fully opened and the contents removed by syringing with ice-cold saline, then gently blotted dry with filter paper and reweighed.

After weighing, the washed intestinal segments were dried at 75° until constant weight (48 h).

Expt 3. To study the effect of feeding RSF on intestinal growth in the rat, four rats fed on RSF and four fed on chow were killed after 48 weeks on these diets. The small intestine was removed *in toto*, the length measured and the gut divided into four segments of equal length. The segments were washed out with cold saline as described previously and weighed.

Expt 4. In view of the unexpected finding of small-intestinal growth in animals fed on RSF, the effect of a pure trypsin inhibitor for up to 8 weeks was studied. Four groups, each of twenty mice, were fed on either (1) stock mouse cubes, (2) RSF, (3) 0·5 g camostate/kg or (4) 2 g camostate/kg. Five animals from each group were killed after 1, 2, 4 or 8 weeks on the diets. All animals were fasted overnight and had free access to water before being killed.

Animals were killed as described for Expt 1. At 1, 4 and 8 weeks a portion of the pancreas was frozen on dry ice for measurement of DNA, RNA and protein later the same day. The small intestine from the pylorus to the end of the ileum was removed, the length measured and the intestine divided into three segments of equal length. The intestinal segments were weighed individually, then flushed with 20 ml ice-cold saline, drained for 15 s, and reweighed.

DNA, RNA and protein assay

About 50 mg frozen pancreas, intestinal mucosa or intestinal wall was homogenized for 1 min in 5 ml ice-cold hypertonic phosphate buffer–saline (0·05 M-Na_2HPO_4–2·0 M-NaCl, pH 7·4) 0·5–1 h after removal. This homogenate was used for the assay of DNA, RNA and protein.

The RNA content was measured using 2·5 ml homogenate by the technique of Dembinski & Johnson (1980) and a portion (50 μl) of the homogenate was assayed for protein using the method of Schacterle & Pollack (1973), with bovine serum albumin in distilled water as a standard.

DNA content was determined by a slight modification of the method of Labarca & Paigen (1980), as follows. The remaining homogenate (2·45 ml) was sonicated for 30 s, and a portion was diluted with hypertonic phosphate buffer (1:50, v/v). Diluted sample (1 ml) was mixed with 1 ml fluorochrome Hoechst 33258 (H33258, Boehringer; 1 μg/ml distilled water) and kept in the dark until read in a fluorimeter with an excitation wavelength of 360 nm and an emission wavelength of 450 nm. Calf thymus DNA (Sigma; 5 μg/ml) in the same buffer was used as the standard.

Statistics

Results are expressed as means with their standard errors. Values for animals fed on differing diets at various times after starting the diets were analysed by analysis of variance, with treatment comparisons by Tukey HSD multiple comparison procedure (Systat MGLH module; Systat Inc; Evanston, IL, USA). Significance was considered to be $P < 0·05$.

RESULTS

Experiment 1

The changes in body weight, pancreatic weight, pancreatic RNA, DNA and protein and small intestinal weight over 24 weeks of feeding RSF are shown in Table 1.

Feeding RSF significantly depressed body weight and increased pancreatic and intestinal weights at all intervals tested. Pancreatic RNA and protein were also increased in mice fed on RSF, compared with those fed on chow, at all time intervals. DNA levels were not significantly different at 1 week or 24 weeks, but were higher in animals fed on RSF at all other times tested.

Duodenal DNA, RNA and protein. The weight, DNA, RNA and protein contents of the mucosa and muscle layers from the proximal 50 mm of the duodenum of animals fed on chow, HSF and RSF for 4 weeks are shown in Table 2.

Feeding RSF significantly increased the weight of both mucosal and muscle layers of the proximal 50 mm of duodenum compared with chow- and HSF-fed animals. In RSF-fed mice, DNA, RNA and protein contents were significantly increased in the mucosal layer, compared with chow-fed animals, and RNA and protein contents were also significantly increased compared with HSF-fed mice. Weight and RNA were increased in the mucosa from animals fed on HSF, compared with control animals. In HSF-fed mice the weight of the muscle layer was significantly less than that of the chow- or RSF-fed animals, and

Table 1. *Body weight, pancreatic weight, pancreatic growth variables and small intestinal weight over 24 weeks of feeding chow and RSF**

(Mean values with their standard errors for four mice† or eight mice‡)

Diet	Time (weeks)	Body wt‡ (g) Mean	SE	Pancreatic wt‡ (g) Mean	SE	RNA† (mg/g pancreas) Mean	SE	DNA† (mg/g pancreas) Mean	SE	Protein† (mg/g pancreas) Mean	SE	Intestinal wt‡ (g) Mean	SE
Chow	1	19·5	0·3	0·24	0·01	4·8	0·3	1·1	0·1	43	3	1·25	0·04
RSF	1	17·1	0·4	0·30	0·02	7·0	0·5	1·2	0·1	63	5	2·02	0·18
Chow	2	23·0	0·7	0·18	0·01	3·3	0·5	1·1	0·1	31	4	1·20	0·10
RSF	2	20·2	0·5	0·45	0·01	10·3	0·3	1·8	0·1	116	5	2·46	0·03
Chow	4	33·5	0·5	0·25	0·01	8·7	0·7	1·9	0·1	46	4	1·39	0·07
RSF	4	26·6	0·6	0·46	0·02	15·2	0·4	2·3	0·1	108	8	2·19	0·06
Chow	8	33·1	0·6	0·27	0·01	10·2	0·6	1·7	0·1	54	4	1·50	0·02
RSF	8	27·5	1·1	0·50	0·02	18·1	1·4	2·1	0·1	116	8	2·37	0·10
Chow	12	33·6	0·3	0·26	0·00	10·5	1·3	1·6	0·1	56	0·6	1·56	0·01
RSF	12	30·1	0·5	0·55	0·02	17·5	0·8	2·1	0·2	119	6	2·43	0·02
Chow	24	42·1	1·6	0·30	0·01	11·5	0·6	2·1	0·1	60	5	1·74	0·09
RSF	24	32·8	1·4	0·56	0·03	16·9	1·4	2·5	0·2	116	13	2·78	0·14

Chow, standard mouse chow; RSF, raw soya-bean flour.

* For details of diets and procedures, see pp. 334–335.

DNA, RNA and protein contents were also significantly less than in RSF-fed animals. However, there was no significant difference between DNA, protein or RNA contents in the muscle layer from chow- and RSF-fed animals.

Histology. In mice fed on RSF for 2 weeks the gut wall was thicker due to an increase in both the villus length and crypt depth (Fig. 1).

Experiment 2

In mice fed on RSF for 3 weeks pancreatic weight (0·547 (SE 0·03) g) was significantly increased compared with mice fed on chow or HSF (0·251 (SE 0·02) and 0·300 (SE 0·01) g respectively; *n* 4 in each group).

After washing out the gut contents, the small intestine remained heavier in RSF-fed animals (2·242 (SE 0·11) g compared with 1·410 (SE 0·09) g for chow-fed animals and 1·462 (SE 0·10) g for HSF-fed animals) but there was no difference in the caecal weight in any group.

When dried to constant weight the dried small intestinal tissue in each case constituted about 20% of the wet weight and the tissue from RSF-fed animals remained significantly heavier than that from the other two groups (0·420 (SE 0·02) g compared with 0·266 (SE 0·02) g for chow-fed animals and 0·240 (SE 0·04) g for HSF-fed animals; *n* 4 in each group).

Experiment 3

In four rats fed on chow or RSF for 48 weeks there was no difference in the intestinal lengths (115 (SE 8) cm compared with 114 (SE 1) cm respectively) or the weights of any of the four segments measured.

Experiment 4

The effects of diets containing camostate for up to 8 weeks on body weight, pancreatic weight and total small intestinal weight are shown in Fig. 2.

Table 2. *Weight, DNA, RNA and protein contents of the mucosa and muscle layers from the proximal 50 mm of the duodenum after 4 weeks on chow, HSF or RSF**

(Mean values with their standard errors for five mice)

			Mucosal layer								Muscle layer							
	Body wt (g)		Wt (mg)		DNA (mg/50 mm)		RNA (mg/50 mm)		Protein (mg/50 mm)		Wt (mg)		DNA (mg/50 mm)		RNA (mg/50 mm)		Protein (mg/50 mm)	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Chow	32·8	0·4	90	6·6	0·28	0·03	0·44	0·03	6·19	0·37	112	7·0	0·55	0·06	0·87	0·09	8·69	0·04
HSF	32·4	0·5	122†	10·4	0·41	0·08	0·75†	0·11	8·05	0·89	79†	7·6	0·48	0·06	0·70	0·06	6·77	0·60
RSF	26·2†‡	0·4	207†‡	20·7	0·67†	0·07	1·25†‡	0·10	12·7†‡	1·73	153†‡	15·6	0·82‡	0·13	1·19‡	0·17	12·78‡	1·53

Chow, standard mouse chow; RSF, raw soya-bean flour; HSF, heated soya-bean flour.

* For details of diets and procedures see pp. 334–335.

† Significantly different from Chow value, $P < 0·05$.

‡ Significantly different from HSF value, $P < 0·05$.

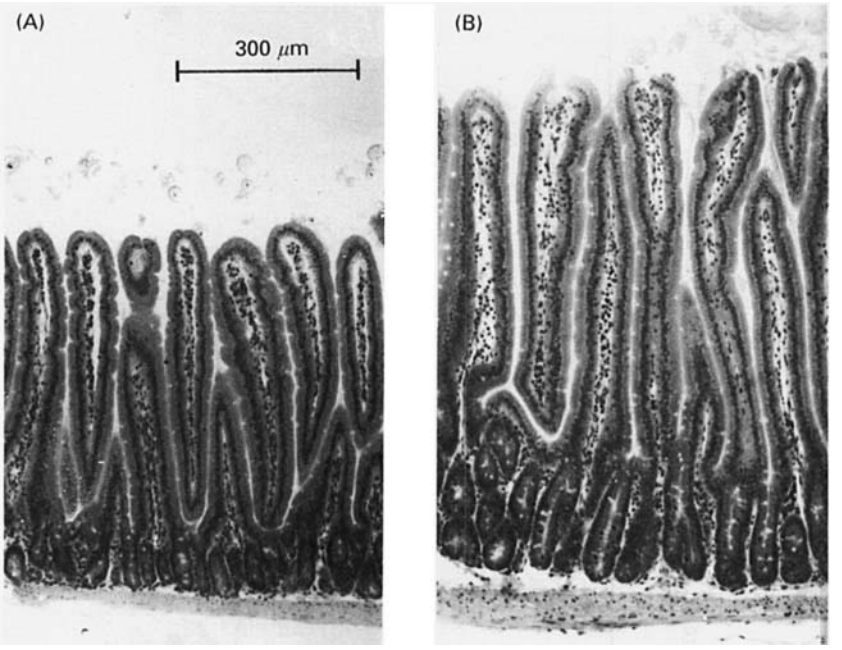

Fig. 1. Duodenal mucosa from a mouse fed on (A) chow or (B) raw soya-bean flour for 2 weeks. Both sections were photographed at the same magnification. The villi and crypts are longer and the muscle wall thicker in the animals fed on RSF.

Body weight. RSF and 2 g camostate/kg diets caused a significant depression of body weight, compared with control animals, at all times studied. From 2 weeks onwards these two diets did not differ significantly in this effect. In contrast, 0·5 g camostate/kg did not affect body weight up to 4 weeks although at 8 weeks body weight was significantly depressed.

Pancreatic weight. Pancreatic weight was significantly increased, compared with control mice, in animals fed on RSF or the camostate diets at all times studied. There was no significant difference between animals fed on RSF or 0·5 g camostate/kg, but from 2 weeks onwards in animals fed 2 g camostate/kg diet pancreatic weight was significantly less than in animals fed on RSF, and at 8 weeks was also less than in animals fed 0·5 g camostate/kg.

Pancreatic growth variables. Pancreatic growth variables (total-pancreatic protein, RNA and DNA) after 1, 4 and 8 weeks on the diets are shown in Fig. 3.

Compared with control animals, at 1 week protein and RNA were increased in animals fed on RSF, but DNA was significantly increased only in animals fed on 2 g camostate/kg. At 2 weeks, the total protein content in the test groups of animals was almost three times the control level and total RNA level was 2·3 times the control values. At this time significant increases in DNA content occurred in animals fed on RSF and 0·5 g camostate/kg, but not in animals fed on 2 g camostate/kg. At 4 and 8 weeks all three variables were increased in animals fed on RSF and 0·5 g camostate/kg, but only RNA was increased in animals fed on 2 g camostate/kg.

Small intestinal weight. Total small intestinal weight (Fig. 2) was markedly increased in animals fed on RSF, compared with all other groups. When compared with chow-fed animals, 0·5 g camostate/kg diet did not affect small intestinal weight, but 2 g camostate/kg diet caused a significant increase in weight at 2 and 8 weeks.

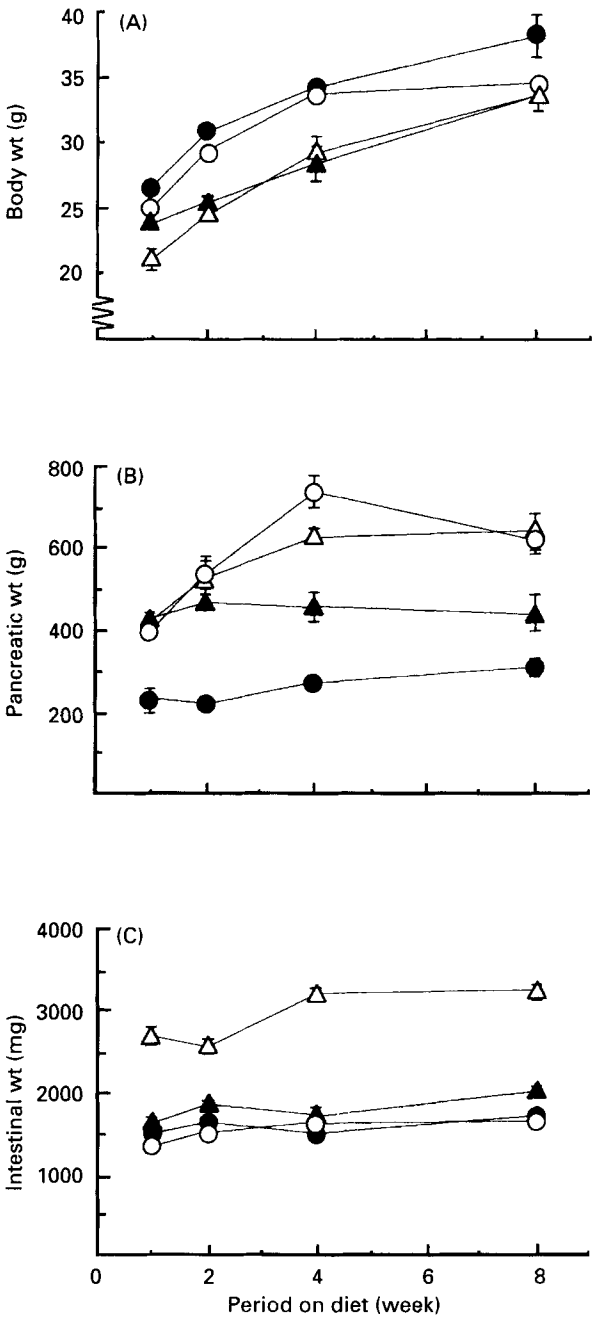

Fig. 2. Changes in (A) body weight, (B) pancreatic weight and (C) intestinal weight in mice fed on chow (●), 0·5 g camostate/kg diet (○), 2 g camostate/kg diet (▲) and RSF (△) for up to 8 weeks. Values are means with standard errors of the means for five mice/group.

In mice fed on RSF, compared with the other diets, there was significant growth of all three segments of the small intestine at all times studied, and this was more marked in the proximal two segments (results not shown). In contrast, compared with chow-fed animals 0·5 g camostate/kg diet did not cause significant growth of any segment of the small

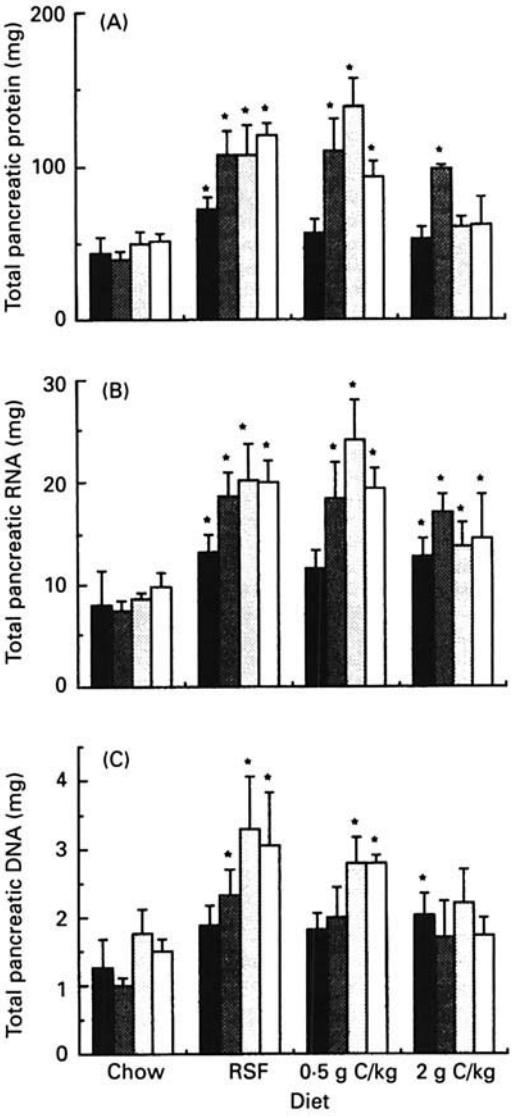

Fig. 3. Total pancreatic content of (A) protein, (B) RNA and (C) DNA in mice fed on chow, RSF, 0·5 g camostate/kg diet or 2 g camostate/kg diet for 1 week ■, 2 weeks ▨, 4 weeks ⊡ or 8 weeks □. n 5 in each group except the group fed 2 g camostate/kg diet for 4 weeks where n 4. *Significantly different ($P < 0·05$) from chow-fed animals at the same time. Error bars indicate SEM. C, camostate.

intestine at any time. In mice fed 2 g camostate/kg diet there was a small, significant increase in weight of the upper third at 2 weeks and the upper and middle thirds at 8 weeks compared with chow-fed animals, but the distal third of the small intestine was not affected.

DISCUSSION

The RSF and chow diets used in the present studies differed significantly in carbohydrate, fat and protein contents. However, HSF did not affect pancreatic and intestinal weights compared with chow (Expt 2), and HSF and RSF have the same composition. It is unlikely,

therefore, that the differences in composition are a significant factor in the intestinal or pancreatic growth seen in animals fed on RSF. There may also have been differences in the amounts of food eaten by animals fed on chow and those fed on RSF and, thus, differences in energy intake. In a separate study with groups of five animals, chow-fed mice ate 5·1 g of food/mouse per d, mice fed on pelleted RSF ate 3·7 g/mouse per d and mice fed on pelleted HSF ate 3·3 g/mouse per d. When the differences in composition of the diets are taken into account these intakes are approximately isoenergetic and the protein intake in animals fed on RSF slightly exceeds that in animals fed on chow. Differences in food intake are, therefore, also unlikely to be significant factors in the differences in intestinal growth.

In the mice fed on RSF for up to 24 weeks pancreatic weight, protein, RNA and DNA all increased, compared with animals fed on chow. In mice fed 0·5 g camostate/kg diet all these pancreatic growth variables also increased at 1, 4 and 8 weeks. Increases in weight, RNA and protein contents are indicators of hypertrophy (cell size), whereas total DNA content is an indicator of hyperplasia (cell number; Melmed *et al.* 1976). The growth of the pancreas in mice fed on RSF and 0·5 g camostate/kg diet, therefore, occurs by both hypertrophy and hyperplasia. These findings confirm previous reports on the effect of camostate in the mouse (Göke *et al.* 1986; Otsuki *et al.* 1987; Wereszczynska-Siemiatkowska *et al.* 1987; Miyasaka *et al.* 1989). In mice fed on 2 g camostate/kg diet all variables were increased after 1 week compared with chow-fed animals, but only weight and total RNA were increased at 4 and 8 weeks. With this larger dose of camostate, therefore, only hypertrophy was seen at the later times. Further studies are needed to define the mechanism for this difference between the two doses of camostate.

Significant growth of the intestinal tract was seen in mice fed on RSF, compared with mice fed on chow, after as little as 1 week. It was confined to the small intestine (Table 2), particularly the upper and middle thirds. In all dietary groups the small intestine contained very little material, so in mice fed on RSF malabsorption, with an increase in the volume of gut contents, was not a significant factor in the increase in small intestinal weight. The weight difference was not due to an increased fluid content of the gut wall since after drying the gut wall to constant weight the small intestine was still significantly heavier in mice fed on RSF. Microscopy of the intestine at 2 weeks (Fig. 1) showed that in the small intestine of RSF-fed mice both the villi and the crypts were enlarged, and the muscle layer appeared thicker. At 4 weeks the RNA, DNA and protein contents of the mucosa were significantly increased (Table 2). The growth of the intestinal mucosa in mice fed on RSF for 4 weeks, compared with mice fed on chow, was therefore by both hypertrophy and hyperplasia.

RSF and dietary trypsin inhibitor stimulate the release of high levels of CCK from the gut wall (Liddle *et al.* 1984; Niederau *et al.* 1987; Roebuck *et al.* 1987). This raised plasma CCK is almost certainly the stimulus for pancreatic growth. It is possible that the raised plasma CCK levels may also be a factor in the growth of the small intestinal wall, but CCK is not usually considered to be trophic for the intestine (Johnson, 1987). Furthermore, this diet had not been reported to affect intestinal growth in the rat, nor was any effect on the growth of the rat intestine seen in the present study (Expt 3). The intestinal growth seen in mice fed on RSF may reflect an indirect effect of TI, since digestion should be delayed in animals fed on RSF and food residue may stimulate gut growth (see p. 343), but little residue was present in the upper small intestine, and no such effect was seen in rats fed on RSF. It seemed more likely that the intestinal response was due to some component of RSF, other than TI, to which the mouse was more sensitive than the rat.

The study with camostate was undertaken to determine whether a synthetic TI, unrelated to the TI present in RSF, could cause growth of both pancreas and gut. The results show that administration of a pure, small molecular weight TI (camostate) at a level of 0·5 g/kg diet causes pancreatic growth comparable to that produced by RSF, but has little effect on

intestinal growth. The TI activity against bovine trypsin of this diet was less than that found in RSF.

A small amount of intestinal growth was seen in mice fed on diets containing 2 g camostate/kg diet, although the growth was significantly less than that seen in animals fed on RSF. This intestinal growth was probably secondary to an increased luminal load, due in turn to maldigestion and malabsorption resulting from inhibition of proteolytic enzymes. Food residues may stimulate gut growth (Johnson, 1987), and intestinal growth and functional small intestinal hypertrophy have been shown in mice with spontaneous exocrine pancreatic insufficiency (although morphological evidence of hypertrophy was not found; Adler *et al.* 1987).

In view of the significantly greater intestinal growth in animals fed on RSF, compared with camostate diets of comparable TI activity, a major part of the effect of RSF must be due to some component other than TI. RSF contains many biologically active components (Liener, 1976), including lectins (Jaffé, 1980). It has recently been shown that, in the rat, lectins from the red kidney bean (*Phaseolus vulgaris*) stimulate small intestinal growth (DeOliveira *et al.* 1988; Tajiri *et al.* 1988) and cause loss of body muscle mass (DeOliveira *et al.* 1988; Bardocz *et al.* 1990). Furthermore, purified soya-bean lectin at a dietary level of 7 g/kg causes intestinal growth in rats (Pusztai *et al.* 1990), although the lectin content of RSF does not appear to be sufficient to cause intestinal growth in this species (Expt 3). Sensitivity to lectins may be species- or even strain-dependent (Jaffé, 1980). The mouse may be more sensitive than the rat to the lectins contained in RSF, and these may, therefore, stimulate small intestinal growth in the mouse, but not the rat, fed on this diet.

Animals fed on RSF and 2 g camostate/kg diet showed a significant depression of body weight, compared with animals fed on chow, at all times studied. This probably reflects in part the maldigestion and malabsorption seen in these animals and the loss of N as a result of markedly stimulated pancreatic secretion (Liener & Kakade, 1980). However, in mice fed on 0·5 g camostate/kg diet no depression of body weight was seen until 8 weeks, despite the fact that pancreatic growth, and presumably pancreatic hypersecretion, was as great as that seen in animals fed on RSF. A considerable part of the early depression of body weight in mice fed on RSF is, therefore, probably due to other active components in this diet, and again lectins may be implicated. As previously mentioned, the kidney bean lectins which cause intestinal growth in rats also cause nutritional problems in that species. Soya-bean lectins may have a similar effect on intestinal growth and nutrition in the mouse.

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