Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract

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Abstract

The lectin, phytohaemagglutinin, present in beans survives passage through the gastrointestinal tract in a biologically and immunologically intact form. It is known that by binding to the brush border membranes of the small intestine phytohaemagglutinin induces its hyperplastic growth. However, its effect on the other parts of the gut are not known. This study considered the dose and time dependent changes in the gastrointestinal tract exposed to phytohaemagglutinin. Lectin binding was detected by polyclonal antibodies using PAP staining to the surface and the parietal cell region of the stomach, the brush border epithelium of the small intestine, and to the surface membrane of the caecum and colon. To characterise the metabolic changes in the gut organ weights, protein, RNA, DNA, and polyamine contents were measured. While phytohaemagglutinin induced a dose and time dependent growth of the small intestine by lengthening the tissue and thickening the gut wall by increasing the number of crypt cells, the lectin also changed the size and metabolism of the large intestine and pancreas, but this growth was by hypertrophy. Phytohaemagglutinin in the diet influences the size, metabolism, and function of the entire digestive tract. The lectin induced changes were fully or partially reversed within three days.

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Raw kidney bean based diets generally reduce the growth rate of animals and induce reversible and polyamine dependent hyperplastic growth of the small intestine in rats.¹⁻⁴ The factor responsible for this is the lectin, phytohaemagglutinin (PHA), present in raw kidney beans.¹⁻³ Most of the dietary proteins are rapidly degraded during passage through the gut by the digestive enzymes present in the small intestine. Undigested nutrients other than the proteins mentioned above, however, are degraded in the caecum and colon by populations of resident bacteria and then used as sources of energy. In contrast with dietary proteins, PHA resists degradation by both proteolytic enzymes and bacteria and keep its biological activity during

intestinal passage in rats.^{5,6} It recognises and binds to complex sugar structures present on brush border membranes of the intestinal epithelium, and as a result, a wide range of local and systemic effects ensue.⁵⁻⁷ One of the most serious consequences of the hyperplastic growth of the small intestine and speeding up of the turnover of brush border cells¹⁻⁴ is that, due to the increase in terminally mannosylated brush border membrane glycans characteristic of immature epithelial cells, there is a dramatic overgrowth of type-1, mannose sensitive fimbriated *Escherichia coli* in the lumen.^{8,9} Also, PHA possesses systemic properties, which result in the atrophy of skeletal muscle and thymus.⁴ Some of the effects are indirect and mediated by PHA induced changes in the hormone balance of the body,^{1,10} while others, at least in part, are caused directly by PHA after its transepithelial transport into the systemic circulation.^{4,5} These effects were found in rats after 7-10 days on kidney bean diets, with a daily intake of about 42 mg PHA. How PHA influences the other parts of the digestive system, namely the stomach, caecum, colon or the pancreas and the liver and what are the consequences of the lectin induced changes for protein, nucleic acid, and polyamine metabolisms in these tissues is less well known. Neither is it clear as to what is the lowest dose of the lectin that can induce gut growth, nor whether a single dose or repeated exposure to PHA will have different effects. Our aims were to study these factors, as the problems have important practical implications for humans. Food aid to Bosnia or Third World countries contains mostly beans as the major source of protein and if the people of these countries cannot afford to cook the beans properly, they are exposed to all the physiological effects of PHA,¹¹ the first site of exposure being the gastrointestinal tract.

Methods

Lectins and other chemicals

Partially purified kidney bean lectin (PHA) preparations were obtained by a simplified procedure described by Pusztai and Watt.¹² The lectin was further purified by affinity chromatography on Fetuin-Sepharose-4B.⁷ Mixed E and L-type PHA isolectins were eluted from the column by 0.05 mol/l glycine-HCl buffer, pH 3.0, containing 0.5 mol/l NaCl. The eluate was dialysed free from salts and recovered by freeze drying after the pH of

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the solution was adjusted to pH 6.8 with 0.05 mol/l NaOH solution. The material obtained from this preparation was used in the experiments.

Animal handling and experimental protocols

Thirty day old Hooded-Lister rats, spf (specified pathogen free) rats of the Rowett colony, weighing 80 ± 1 g, kept singly in cages were pre-fed ($6 \text{ g rat}^{-1} \text{ day}^{-1}$) a semi-synthetic, good quality diet containing lactalbumin (100 g/kg diet) as the sole protein source⁸ for three days. Groups of rats (five animals per group) were then fed the experimental diets (see experiments 1–4) for up to 10 days. On the morning the rats were to be killed, the animals fed PHA containing diets were intra-gastrically intubated with half their daily dose of purified PHA dissolved in saline. Control rats were intubated with saline solution only. Rats were killed by halothane overdose two hours after intubation and their stomach, jejunum (5–25 cm from pylorus), the remaining parts of the small intestine, caecum, colon, pancreas, and liver were removed, washed with ice cold saline, blotted dry, and weighed. Part of the small intestine (2 cm; 5 cm from the pylorus), together with representative sections of the pancreas, stomach, colon, and caecum were cut, weighed, and fixed in 4% phosphate buffered (pH 7) formalin for histological examination. The remainder of the tissues was freeze dried to constant weight and used for chemical analyses.

Experiment 1 – rats (groups of five were fed 6 g/day lactalbumin (control) diet with or without the inclusion of purified PHA ($42 \text{ mg rat}^{-1} \text{ day}^{-1}$) for 10 days.

Experiment 2 – to establish the time dependent changes induced by PHA, rats (groups of five) were given either 6 g of the lactalbumin diet or a lactalbumin and kidney bean protein based feed, which contained $42 \text{ mg PHA rat}^{-1} \text{ day}^{-1}$ for 0, 1, 3, 5, 7 or 10 days.

Experiment 3 – the dose effect of PHA was established with groups of rats, pair fed either on the control diet ($6 \text{ g rat}^{-1} \text{ day}^{-1}$) or on diets containing 0.65, 1.3, 2.6, 5.2, 10.5, 15, 21, 30 or $42 \text{ mg PHA rat}^{-1} \text{ day}^{-1}$ respectively for 10 days.

Experiment 4 – five rats were fed on 6 g/day lactalbumin diet for six days (control group). A second group of five rats was given the control diet for three days then changed to a PHA containing diet ($42 \text{ mg PHA rat}^{-1} \text{ day}^{-1}$) for three days. The third group (five rats) was given the lectin diet for three days followed by the control diet for three days. All rats were killed at the end of the six day period.

Chemical analysis

Tissue samples were extracted in the presence of an internal standard of 1,7-diamino heptane with 10% (w/v) perchloric acid (15 mg tissue/ml) for 30 minutes at 0°C and then centrifuged. Individual polyamine concentrations in the supernatant were analysed by high performance liquid chromatography.¹³

The protein content of the residue insoluble in perchloric acid was determined by a modified Lowry method¹⁴ after solubilisation in 0.3 mol/l NaOH. RNA¹⁵ and DNA¹⁶ concentrations were estimated as described previously.⁸

Histological assessment

Sections of different parts of the digestive tract were fixed in 4% (w/v) buffered (pH 7) paraformaldehyde, embedded in paraffin wax, and slices ($3 \mu\text{m}$) were stained with haematoxylin and eosin for morphological measurements. Ten properly oriented villi and crypts from the jejunum and other parts of the gastrointestinal tract were selected at random from each animal and their length measured. Results were calculated as mean (SD) of five rats per treatment group.

Antibody peroxidase antiperoxidase (PAP) staining of the sections was carried out as before.⁶ Briefly, after inhibition of the endogenous peroxidase in fixed tissue sections, antigenic sites were unmasked by trypsinisation and the sections were reacted with appropriate anti-PHA antibodies in the presence of fetuin, followed by the link antiserum and then PAP serum. The bound PHA lectins were then visualised with 3,3'-diaminobenzidine and the sections were counterstained with haematoxylin.

Statistical analysis

The results were subjected to one way analysis of variance (ANOVA) using the Minitab computer program (Scottish Agricultural Statistics Service, Edinburgh, UK). When *p* values were <0.05 , the significance between groups was estimated by Student's *t* test.

Results

Effect of $42 \text{ mg PHA rat}^{-1} \text{ day}^{-1}$ for 10 days on the rat gastrointestinal tract

Table I shows changes in the wet weight and dry weight (expressed as a percentage of the

TABLE I Effect on the weights of the gastrointestinal tract, pancreas, and liver of rats after treatment with PHA for 10 days

Organs	PHA	Lactalbumin
Wet weight (mg)		
Stomach	588 (42)	666 (17)†
Jejunum (20 cm)	1309 (58)	1070 (49)†
Small intestine	6255 (294)	4308 (78)†
Caecum	559 (21)	470 (5)†
Colon	546 (78)	471 (16)
Pancreas	624 (60)	442 (22)†
Liver	3047 (183)	3554 (297)†
Dry weight/dry body weight*		
Stomach	0.61 (0.05)	0.50 (0.01)†
Jejunum (20 cm)	1.30 (0.05)	0.61 (0.05)†
Small intestine	5.57 (0.19)	2.44 (0.08)†
Caecum	0.51 (0.05)	0.34 (0.03)†
Colon	0.54 (0.09)	0.35 (0.02)†
Pancreas	0.79 (0.08)	0.42 (0.03)†
Liver	4.46 (0.21)	3.74 (0.25)†
*Dry body weight (g)	19.3 (1.1)	28.5 (1.2)†

†Significantly different from the PHA fed rats ($42 \text{ mg PHA rat}^{-1} \text{ day}^{-1}$) at $p < 0.05$. The values represent the mean (SD) for five rats/group.

TABLE II Chemical composition of the gastrointestinal tract of rats after 10 days on a PHA diet compared with those fed a lactalbumin (La) diet (control)

	Protein (mg)	RNA (mg)	DNA (mg)	Putrescine (nmol)	Spermidine (nmol)	Spermine (nmol)
<i>Stomach</i>						
La	95 (3)	3.3 (0.1)	0.7 (0.1)	9 (3)	351 (43)	255 (38)
PHA	82 (3)*	2.7 (0.1)*	0.5 (0.1)*	8 (2)	335 (30)	257 (24)
<i>Jejunum (5–25 cm)</i>						
La	100 (4)	3.6 (0.4)	2.9 (0.2)	47 (18)	499 (33)	385 (14)
PHA	134 (8)*	8.3 (0.6)*	3.6 (0.2)*	238 (32)*	677 (11)*	471 (11)*
<i>Small intestine</i>						
La	449 (12)	7.7 (1.8)	6.8 (0.5)	277 (41)	1764 (43)	1367 (25)
PHA	667 (72)*	16.3 (1.2)*	9.5 (1.2)*	1047 (366)*	2551 (29)*	2284 (153)*
<i>Caecum</i>						
La	58 (2)	1.8 (0.1)	1.4 (0.1)	11 (3)	316 (32)	191 (18)
PHA	65 (5)*	3.1 (0.6)*	1.4 (0.1)	25 (9)*	457 (89)*	253 (27)*
<i>Colon</i>						
La	57 (3)	1.8 (0.2)	1.5 (0.3)	8 (3)	334 (16)	235 (16)
PHA	63 (6)	3.1 (0.3)*	1.6 (0.4)	45 (9)*	451 (145)	299 (74)
<i>Pancreas</i>						
La	79 (2)	3.9 (0.3)	0.9 (0.1)	11 (3)	921 (14)	310 (33)
PHA	92 (8)*	7.4 (2.2)*	0.9 (0.1)	26 (12)*	1147 (73)*	395 (57)*

*Significantly different from the control rats at $p < 0.05$. Values represent mean (SD) for five rats/group. Rats were fed on diets providing 42 mg PHA $\text{rat}^{-1} \text{ day}^{-1}$.

body weight) of the different parts of the gastrointestinal tract of rats from experiment 1.

Table II shows changes in metabolic activity of the tissues, as shown by their protein, RNA, DNA, and polyamine (putrescine, spermidine, and spermine) concentrations. Significant changes were seen both in the size and chemical compositions of all organs examined. The wet weight and the dry tissue weight/body weight ratios of every part of the digestive tract increased when PHA was included in the diet (Table I). Based on the increase in DNA and spermine contents, the growth of the jejunum and of the small intestine was by hyperplasia, which is in agreement with earlier findings.^{1–4} The increase in colonic and pancreatic weight was by hypertrophy, as the DNA concentrations did not change significantly (Table II). The absolute weight of the stomach and liver were slightly reduced, but this was proportionally less than the loss of body weight (Table I).

Binding, time and dose dependent changes induced by PHA

Stomach – after feeding phytohaemagglutinin containing diets to rats for three days, the lectin was bound to the surface epithelium and the parietal cell region of the stomach (Fig 1A). On changing the animals back to lectin free (control) diet for three days, the surface binding disappeared, but the lectin could still be detected in the parietal cells (Fig 1B). We also observed that stomach emptying was much slower in PHA treated rats than in controls. Treatment with the highest dose of PHA (42 mg $\text{rat}^{-1} \text{ day}^{-1}$) for three days temporarily increased the length of gastric pits, and the putrescine and DNA contents of the stomach tissue (Tables III and IV). However, the weight and the protein, RNA and DNA contents of the stomach of rats fed for 10 days with diets containing this highest dose of PHA became significantly reduced in comparison with control rats (Tables I and II). This occurred without significant changes in the polyamine content of gastric tissues. Moreover at lower doses, none of the weight or compositional changes reached significance (results not given).

Figure 1: Binding of PHA to the stomach of rats fed (A) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ providing 42 mg PHA for three days followed by (B) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ without PHA for three days. PHA was visualised by a polyclonal PHA-antibody detected by PAP staining (magnification: 80×3.4).

Small intestine – the growth factor like effect of PHA, resulting from the strong binding to the brush border membrane (Fig 2A), increased both the length and the thickness of the small intestine (Fig 3 and Table III). However, the dose and time dependent changes induced by PHA are novel. The changes in the jejunum were more pronounced than in the ileum. The small intestinal length increased continuously with increasing doses of PHA and had become significantly longer by

day 3 (Fig 3). The dry weight showed a similar trend up to day 7, after which it seemed that the growth levelled off (Fig 4). With longer treatment there was no further increase, but rather a decrease in all parameters measured. A nearly linear relation between the weight of the tissue and the dose of PHA was seen between doses 0.65 and 30 mg PHA rat⁻¹ day⁻¹ (Fig 5). Increasing the dose to 42 mg PHA rat⁻¹ day⁻¹ had no further effect. Within three days of removing PHA from the diet of rats, the protein and DNA concentrations of the jejunum (Table IV) returned to values measured in controls but the length of the crypt, the weight and RNA content, though decreased significantly, were not yet fully restored to normal (Table III).

Large intestine – binding of PHA to the surface of both the caecum (Fig 6A) and colon (Fig 7A) was detected by immunohistochemical methods. As there was an increase in weight, protein, RNA, putrescine, and spermidine concentrations, it would seem that the metabolic activity in the large intestine was increased by PHA (Tables II and IV). No significant changes were seen in growth during the first three days of PHA treatment, but with longer treatment a continuous increase was detected (Fig 4). Low doses of PHA had no effect, but increasing the dose to 10.5 mg PHA rat⁻¹ day⁻¹ or above, slightly increased the tissue weight. However, above

10.5 mg PHA rat⁻¹ day⁻¹, no further increases were seen after 10 days (Fig 5). When PHA was removed, most of the PHA induced changes were reversed (Tables III

TABLE III Histological analysis of the different parts of the gastrointestinal tract (length, µm) of rats fed either lactalbumin or PHA containing diets, or both, for six days

	Stomach crypt	Jejunum (5–7 cm‡)		Caecum crypt	Colon crypt
		Villus	Crypt		
La	435	428	117	186	206
La/PHA‡	474*	509*	283*	215*	280*
PHA/La	406†	490†	157†	200†	194†
Pooled SD	4	9	4	4	5

Values are means for five rats. Significantly different at p<0.05 from *controls and ‡La/PHA. ‡5–7 cm from the pylorus. La=lactalbumin diet only (controls). La/PHA=lactalbumin diet for three days followed by PHA containing diet (42 mg PHA rat⁻¹ day⁻¹) for three days. PHA/La=PHA containing diet (42 mg PHA rat⁻¹ day⁻¹) for three days followed by lactalbumin diet for three days.

TABLE IV Chemical composition of the gastrointestinal tract of rats fed lactalbumin or PHA containing diets, or both for six days

	Dry weight (mg)	Protein (mg)	RNA (mg)	DNA (mg)	Putrescine (nmol)	Spermidine (nmol)	Spermine (nmol)
<i>Stomach</i>							
La*	123	69	3.3	0.9	14	317	332
La/PHA†	131	71	3.8	1.3*	138*	360	335
PHA/La‡	118†	70	3.3	0.9†	47†	297†	307
Pooled SD	8	11	0.5	0.1	15	40	26
<i>Jejunum</i>							
La	126	84	3.7	1.9	35	567	339
La/PHA	188*	107*	11.8*	3.1*	318*	1130*	502
PHA/La	165†	84†	5.4*†	2.0†	74*†	668*†	485*
Pooled SD	12	10	1.0	0.3	34	70	21
<i>Caecum</i>							
La	87	49	1.9	1.1	125	234	278
La/PHA	119*	68*	4.6*	1.7*	369*	376*	355*
PHA/La	91†	44	2.3†	1.1†	149†	260†	248†
Pooled SD	14	9	0.5	0.2	37	33	40
<i>Colon</i>							
La	118	49	1.6	1.4	24	219	223
La/PHA	114	72*	3.7*	1.7	265*	513*	355*
PHA/La	122	54†	1.7†	1.7	55*†	224†	247*†
Pooled SD	27	7	0.5	0.2	21	29	18

Values are means of five rats. Significantly different at p<0.05 from *controls and ‡La/PHA. La=lactalbumin diet only (controls). La/PHA=lactalbumin diet for three days followed by PHA containing diet (42 mg PHA rat⁻¹ day⁻¹) for three days. PHA/La=PHA containing diet (42 mg PHA rat⁻¹ day⁻¹) for three days followed by lactalbumin diet for three days.

Figure 2: Binding of PHA to the jejunum of rats fed (A) 6 g lactalbumin diet rat⁻¹ day⁻¹ providing 42 mg PHA for three days followed by (B) 6 g lactalbumin diet rat⁻¹ day⁻¹ without PHA for three days. PHA was visualised by a polyclonal PHA-antibody detected by PAP staining (magnification: 100×3.4).

Figure 3: The effect of PHA fed over 10 days and in increasing amounts on the length of the rat small intestine.

and IV). Even after three days, however, the presence of some PHA was detected on the brush border membrane of cells at the bottom of the crypt in both the caecum and colon (Figs 6B and 7B).

Pancreas – PHA induced hypertrophy of the exocrine pancreas, which was evident from the increase in size of pancreatic islets.¹⁷ Generally, a continuous, dose and time related hypertrophic growth of the pancreas was seen in these experiments (Figs 4 and 5). The lowest doses (up to 7 mg PHA rat⁻¹ day⁻¹) studied, however, had no effect after 10 days and maximal effect was achieved by 21 mg PHA rat⁻¹ day⁻¹. Further increase of the dose had no further effect (Fig 5). The PHA induced growth of the pancreas was evident from day 3 and increasing the exposure time from seven to 10 days on 42 mg PHA rat⁻¹ day⁻¹ did not increase pancreatic dry weight further (Fig 4).

Liver – there was a significant reduction in

liver weights of rats exposed to PHA over the 10 day experimental period (Fig 4). The changes in liver weights did not follow any trend with the different doses of PHA (Fig 5). When tissues were analysed for composition, variations between treatment groups were smaller than changes within experimental groups.

Discussion

The growth factor-like effect of PHA,^{3,4,8} or diets containing raw kidney bean proteins^{1-4,6,7} is well established in both germ free rodents (rats and mice) and those with a conventional microflora. It has been known for some time that the most profound effect of PHA is on the small intestine^{1-8,18,19} or Caco-2 cells, which are regarded as appropriate in vitro models of small intestinal epithelial cells,²⁰ and that the lectin has a greater effect on the jejunum than the ileum.¹⁻⁴ The trophic effect of PHA on the small intestine can be explained by its strong binding to the complex glycosyl side chains occurring on the surface of the brush border membrane glycoconjugates (Figs 1 and 2;^{1,6}).

As neither the stomach nor the large intestine have villi, binding of the lectin to these parts of the digestive tract has not been investigated. It was also thought that the acidic conditions in the stomach should prevent the lectin from binding to its surface epithelium. As we have previously seen, however, that PHA induces changes in the function and metabolism of the entire digestive tract of rats, our goal was to systematically study the binding of PHA to each part of the gastrointestinal tract and to see the effect of this binding on metabolic activity.

We observed that the PHA diet slowed down stomach emptying. At the time of killing (two hours after the last food intake) the stomach of all rats given PHA was full and distended, but not that of the lactalbumin fed rats. Furthermore, when the pH of the stomach content was measured in PHA fed animals, there was a significant loss of acidity,

Figure 4: Changes in the dry weight of the small intestine, large intestine, pancreas, and liver of rats given 6 g lactalbumin diet rat⁻¹ day⁻¹ providing 42 mg PHA rat⁻¹ day⁻¹ for 10 days.

Figure 5: Changes in the dry weight of the small intestine, large intestine, pancreas, and liver of rats given 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ providing 0, 0.65, 1.3, 2.6, 5.2, 10.5, 15, 21, 30, or 42 mg PHA $\text{rat}^{-1} \text{ day}^{-1}$ for 10 days.

as the pH in the stomach had increased to 5.6–6.2 whereas in the control animals the pH was 2.5–3.5. Using a polyclonal PHA antibody, it became evident that PHA was extensively bound to the surface epithelium and the parietal region of the gastric pits. Three days after returning rats to a lectin free diet, PHA was still detected in the parietal cell region. These findings show that, while the surface layer of the stomach epithelium is renewed within three days, the specialised parietal cells are not. It is probable that PHA binding to these cells inhibits either gastric acid secretion or the functioning of the proton pumps, both of which may lead to impaired acid secretion and a consequent increase in stomach pH. The strong binding of PHA to the brush border membrane of the small intestine (Fig 1B;^{6,17}) resulted in a polyamine dependent,^{1,2} hyperplastic and hypertrophic growth of the tissue. However, in addition to the increase in crypt cell numbers, resulting from increased mitotic activity,³ there was a definite, time and dose dependent increase in the length of the tissue (Figs 4 and 5;³). On feeding rats 15 mg PHA $\text{rat}^{-1} \text{ day}^{-1}$ for up to seven days, the small intestine lengthened continuously, reaching 94.8 (2.5) cm from 84 (2) cm. With a higher dose (42 mg PHA $\text{rat}^{-1} \text{ day}^{-1}$) the growth was even more extensive as the length had increased to 100 (2) cm by day 5. When the lectin was removed from rat diets, the staining of the brush border membrane of the small intestine with anti-lectin antibodies disappeared, indicating that practically all the cells that were in contact with PHA were replaced during the three day period, and the entire surface of the small intestine was now covered with new cells.

PHA bound to the epithelium of the upper part of the gastrointestinal tract is liberated when villus tip cells are shed into the gut lumen. As all cellular material, except the lectin, will be removed by digestion,⁶ PHA becomes free to roll down to more distal parts

Figure 6: Binding of PHA to the caecum of rats fed (A) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ providing 42 mg PHA for three days followed by (B) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ without PHA for three days. PHA was visualised by a polyclonal PHA-antibody detected by PAP staining (magnification: 100×3.4).

of the bowel and bind again to the next suitable carbohydrate structure. Therefore, PHA was bound to the surface of not only the small but also the large intestine and affected its

Figure 7: Binding of PHA to the colon of rats fed (A) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ providing 42 mg PHA for three days followed by (B) 6 g lactalbumin diet $\text{rat}^{-1} \text{ day}^{-1}$ without PHA for three days. PHA was visualised by a polyclonal PHA-antibody detected by PAP staining (magnification: 100×3.4).

metabolism. As significant increases of both the caecal and colonic tissues were not evident before day 5 of PHA treatment, this delay might reflect the time lag for the lectin

to reach the large intestine and begin to induce changes in cell metabolism.

The delay was also evident from the fact that after three days on a lectin free diet, the presence of PHA could still be detected in the lower parts of the caecal and colonic crypts.

It is also known that PHA is partially endocytosed (Fig 1B;⁴⁻⁶) and affects the size and function of the pancreas by an unknown mechanism. The increase in the size of pancreatic acini¹⁷ and the chemical composition of the tissue pointed to hypertrophy. There were no changes in DNA content, but protein, RNA, putrescine, spermidine, and spermine concentrations were all significantly increased, pointing to increased metabolic activity of the tissue (Table II). It has also been established, that this hypertrophy of the pancreas affects its function, or at least the synthesis or secretion of insulin, or both by the islets.¹⁰

The increased metabolic activity of the gastrointestinal tract puts pressure on the liver. Despite a reduction in the liver weight of rats treated by the lectin, the proportional weight of the liver increased with treatment time as the reduction of body mass was more intensive than the loss of liver tissue.

In summary, it was established that PHA binds to the surface layer of the entire digestive tract including the stomach, small, and large intestines. The consequences of this binding were organ dependent: the growth of the small intestine was by hyperplasia while that of the caecum, colon, and pancreas was by hypertrophy, but metabolic activity increased in all parts examined. The lectin induced changes were time and dose dependent, and were also reversible. When the growth signal, PHA, was removed, all changes were either fully or partially reversed.

In countries stricken by famine or war, beans are an important source of proteins, because they are easy to store. However, as all bean samples examined by us, except Pinto III,^{21 22} contained significant amounts of PHA, before they could be safely consumed they must be properly cooked. To eliminate the growth factor-like and anti-nutritional effects of the lectins in the beans requires boiling the beans for at least 10–15 minutes. The population of these countries, however, are not only short of food, but they are also short of firewood and other sources of energy. It is therefore of fundamental importance to establish the exact consequences on the gastrointestinal tract and other organs of the body of ingesting partially cooked or raw bean proteins.

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