

Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects

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Experiments were designed to investigate whether orally consumed tomato lectin could resist the digestive process and function as a lectin within the alimentary canal. Rats fed on a tomato lectin-rich diet passed faeces containing serologically detectable tomato lectin, and the lectin could be shown by immunoperoxidase staining bound to intestinal villi. Moreover, radioactivity was mainly recovered from the alimentary canal 3h after ¹²⁵I-labelled tomato lectin administration with only traces in the circulation or internal organs. Radioactivity absorbed into the human circulation after consumption of ¹²⁵I-labelled tomato lectin was also less than that expected for a digestible protein.

Tomato lectin Dietary lectin Villus structure Rat intestine Human intestine

1. INTRODUCTION

Many foodstuffs contain lectins [1], although the biological activity of dietary lectins is usually destroyed by cooking or autoclaving. Little is known about the consequences of consuming lectins in a native form, except for phytohaemagglutinin (PHA), the lectin from kidney beans (*Phaseolus vulgaris*). Uncooked or partly cooked kidney beans cause severe enteritis in humans after a single dose, and rats fed on diets containing kidney bean meal die within a few days [2-4]. This toxicity is largely due to PHA [5], and recently there has been concern because some 'starch-blocker' slimming tablets contain PHA, and therefore may be dangerous [6,7]. Studies of rats fed PHA-containing diets have revealed that the lectin binds to intestinal villi, leading to disruption of the villus structure [8]. Unlike kidney beans,

tomato (*Lycopersicon esculentum*) fruits are often consumed raw or lightly cooked, and therefore a greater quantity of tomato lectin [9,10] is probably consumed in an active form than that of any other known dietary lectin. This work was undertaken to try to establish whether orally consumed tomato lectin could function as a lectin within the gastrointestinal tract. We provide evidence that the tomato lectin resists digestion in the alimentary canal of rats, and binds to rat intestinal villi without causing disruption of their integrity. Experiments on a human subject indicate that analogous results might be expected in humans. Dietary consumption of tomato fruits might have important biological consequences which could be exploited for therapeutic use.

2. MATERIALS AND METHODS

2.1. Lectins and antiserum

Tomato lectin and rabbit anti-(tomato lectin) serum were prepared as described [11]. Iodinated

Abbreviations: BSA, bovine serum albumin, PHA, phytohaemagglutinin; WGA, wheat germ agglutinin

(^{125}I) tomato lectin was prepared by reacting 0.25 ml purified lectin in phosphate-buffered saline (12 mg/ml) with 1 mCi Na^{125}I in the presence of 4 Iodobeads (Pierce) as described in [12]. WGA was purchased from Sigma.

2.2. Diets

One diet was prepared of which 50% (w/w) was lyophilised tomato juice, a rich source of tomato lectin [9], contributing half of the protein content of the diet. The remainder of the protein was supplied by casein (5% by wt); the diet also contained the required starch, glucose, oil, minerals and vitamins as described [13,14]. Two control diets were also prepared: one with 10% casein and no tomato extract; and a non-protein diet with starch in place of the casein.

2.3. Studies in rats using ^{125}I -labelled tomato lectin

Tomato lectin was iodinated with Na^{125}I , repurified by affinity chromatography to ensure only biologically active material was used, then dialysed extensively against physiological saline. The labelled lectin was then diluted into fresh tomato juice to give a radioactivity of about $1\ \mu\text{Ci}/\text{ml}$. BSA (50 mg/ml) was then added. This preparation (1 ml) was introduced by tube into the stomach of Lister rats (weighing approx 100 g), which had been fasted overnight. Immediately afterwards, two rats were injected with a potentially lethal dose of pentobarbitone solution ('Anasleep Forte', Dales Pharmaceuticals), and when death appeared imminent, a blood sample was obtained by cardiac puncture. The alimentary canal was clamped at the distal end of the oesophagus, at the pylorus, and just before the caecum. Selected internal organs were excised and frozen immediately in dry ice. The stomach was separated from the small bowel and its contents washed out with ice-cold 0.05 M sodium phosphate, pH 7.8. The intestinal contents were washed out with ice-cold 0.1 M citric acid/0.2 M sodium phosphate, pH 3.0. The stomach and intestinal contents were then diluted with an equal volume of 30% trichloroacetic acid. The internal organs were thawed out in 30% trichloroacetic acid, chopped up with scissors, then homogenised in trichloroacetic acid solution using a top-drive tissue homogeniser. This procedure was repeated

at 1.5 and 3 h after the administration of the ^{125}I -lectin-containing meal. The total radioactivity was measured using an ICN Tracerlab Spectomatic gamma counter, before insoluble material was collected by centrifugation and the supernatant discarded. After washing the precipitate again in trichloroacetic acid, the trichloroacetic acid-insoluble material was counted for radioactivity.

2.4. Immunoperoxidase staining of thin sections

Gut segments were fixed in 0.9% saline containing 10% formalin and 2% acetic acid for 16 h at 20°C , embedded in paraffin, and sectioned at $8\ \mu\text{m}$. The sections were treated with rabbit (anti-tomato lectin) serum or pre-immune serum followed by swine anti-rabbit immunoglobulin then peroxidase-anti-peroxidase prepared in rabbits. The immunochemical reagents were used at optimal dilutions, and were applied to the sections for 30 min followed by a 30 min wash in 0.9% saline. Finally, peroxidase activity was visualised with diaminobenzidine in Tris buffer, pH 7.6, for 10 min before counterstaining with haematoxylin.

3. RESULTS AND DISCUSSION

3.1. Rat feeding experiments

A tomato lectin-rich diet, a casein control diet and a non-protein control diet were prepared. Groups of 4 rats were fed the diets for 10 days. The rats fed on 5% tomato protein/5% casein lost considerably less weight than those fed on the protein-free diet and gave a net protein utilisation value of 23. This was close to that predicted (26) from the chemical score [15]. This experiment therefore demonstrated that the tomato lectin, unlike PHA, is non-toxic for rats.

Faeces of rats fed on the tomato extract diet were collected, and extracted in phosphate-buffered saline. This faecal extract formed a weak line of precipitation after Ouchterlony double diffusion against rabbit anti-(tomato lectin) serum, forming a reaction of identity with authentic tomato lectin in an adjacent well, but did not react with pre-immune serum from the same rabbit. Some tomato lectin could therefore survive passage through the alimentary canal of a rat in a form serologically indistinguishable from the native lectin.

3.2. Recovery of radioactivity from rats given ^{125}I -labelled tomato lectin

To study the fate of ingested tomato lectin more precisely, fasted rats were fed a meal containing $1\ \mu\text{Ci}$ ^{125}I -labelled tomato lectin in BSA-enriched tomato juice. At intervals the rats were killed, dissected and the total and trichloroacetic acid-precipitable radioactivity in each organ counted (fig.1 and table 1). The zero time referred to was actually about 10 min after the administration of labelled lectin, the time required for the anaesthetic to take effect, and a blood sample to be taken from the heart. The lectin was found to be very resistant to the digestive process. After 3 h, by which time most of the lectin had passed through the stomach, and a significant proportion had reached the colon, only about 3% of the radioactivity administered was recovered from the internal organs and serum. The remainder was recovered from the alimentary canal, and about 50% of it was in a trichloroacetic acid-precipitable form. Within the stomach, little breakdown was apparent even after 3 h. Only 5–9% of the radioactivity in the stomach was associated with gastric tissue at any time, virtually all of it trichloroacetic acid-precipitable. Over 90% of the radioactivity in the stomach was washed out with the contents, and even after 3 h, 59% of that radioactivity was trichloroacetic acid-precipitable. Within the small intestine, 62% of the radioactivity was tissue-

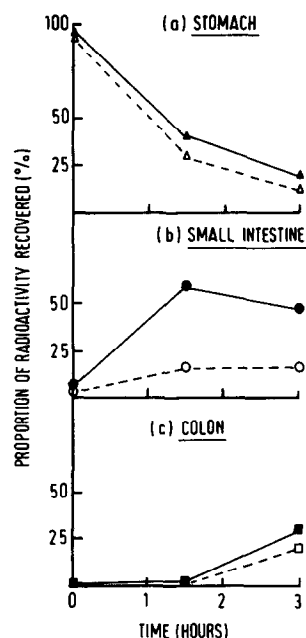


Fig.1. Recovery of radioactivity from the alimentary canal as a function of time after administration of ^{125}I -labelled tomato lectin. Closed symbols, mean cpm of two rats, expressed as a proportion of the total radioactivity recovered, open symbols, proportion of the total radioactivity recovered that was trichloroacetic acid-precipitable. The recovery of radioactivity administered was quantitative, and >97% was present in the alimentary canal at the times chosen.

Table 1

Distribution of radioactivity in selected rat internal organs 3 h after administration of ^{125}I -labelled tomato lectin into the stomach

Organ	Total cpm	Trichloroacetic acid-precipitable cpm	% total radioactivity	% total radioactivity per g wet wt
Liver	10999	5777	1.02	0.13
Kidneys	3200	789	0.30	0.21
Lungs	1200	390	0.11	0.10
Spleen	590	142	0.06	0.12
Thymus	580	92	0.05	0.08
Pancreas	430	90	0.04	0.09
Serum (0.2 ml)	240	23	0.20	—
Serum (8 ml)	9600	920	0.90	—

For experimental details, see section 2. Results are expressed as mean values obtained from two rats after the deduction of background counts. Total serum cpm has been calculated by assuming a volume of 8 ml for the size of rats used.

Table 2

Excretion of radioactivity after administration of ^{125}I -labelled tomato lectin

Source	cpm (faeces)	cpm (urine)
^{125}I -labelled tomato lectin	232559 (21%)	873700 (79%)
^{125}I -BSA	16944 (1%)	1387684 (99%)
Na^{125}I	15351 (1%)	1257987 (99%)

Rats were fed by stomach tube approx $1\ \mu\text{Ci}$ ^{125}I -labelled tomato lectin, ^{125}I -BSA or Na^{125}I in 1 ml tomato juice containing 50 mg BSA. The BSA (at 4 mg/ml) was iodinated in the same way as tomato lectin [12]. The urine and faeces from each rat were collected during the following 48 h. The recovery of radioactivity in excretion products was quantitative, less than 0.03% of the radioactivity administered was present in the thyroid at 48 h. Results given are mean values obtained from two rats.

associated at zero time, all of it trichloroacetic acid-precipitable; however, at later times, 90% of the radioactivity became detached from the gut wall. Always, the tissue bound radioactivity in the small intestine was significantly more trichloroacetic acid-precipitable than that of the intestinal contents: 100% vs 37% at zero time, 78% vs 24% at 1.5 h, 60% vs 31% at 3 h. We interpret these figures to mean that on entering the small bowel, the lectin binds to the gut wall until all the binding sites are occupied. Bound lectin is then protected (relative to free lectin) from proteolytic enzyme attack and is degraded more slowly. Alternatively, only intact lectin can bind to the gut wall and when broken down it is replaced by intact lectin from the gut lumen. Either explanation is consistent with the immunohistochemical studies (see below).

Our conclusion that tomato lectin is not fully digested in the rat alimentary tract was confirmed by an alternative approach. Rats were fed either

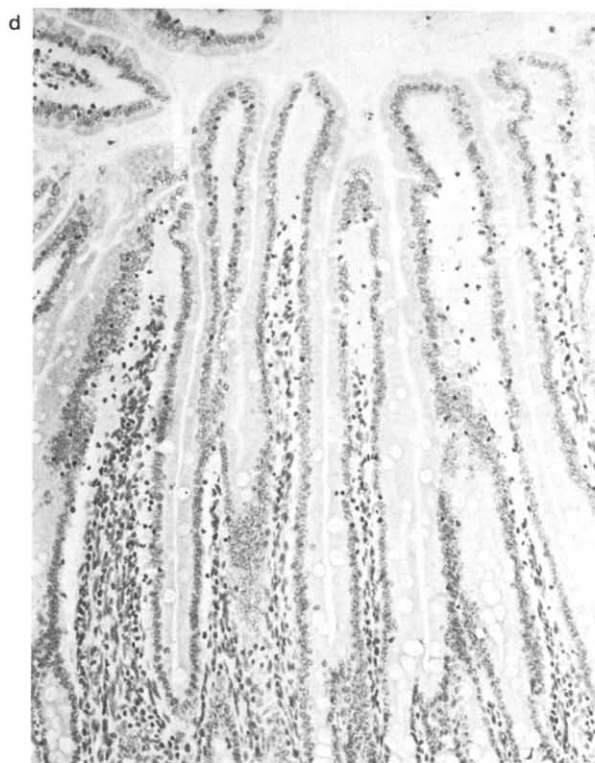
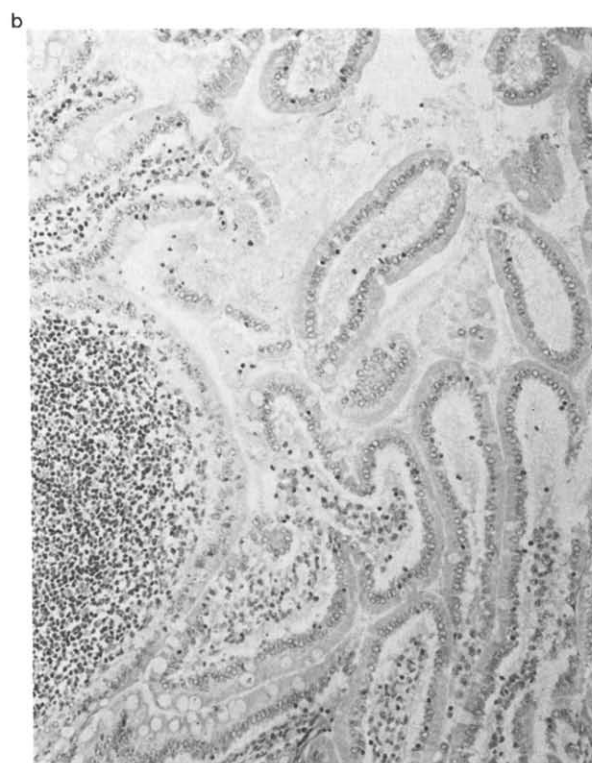
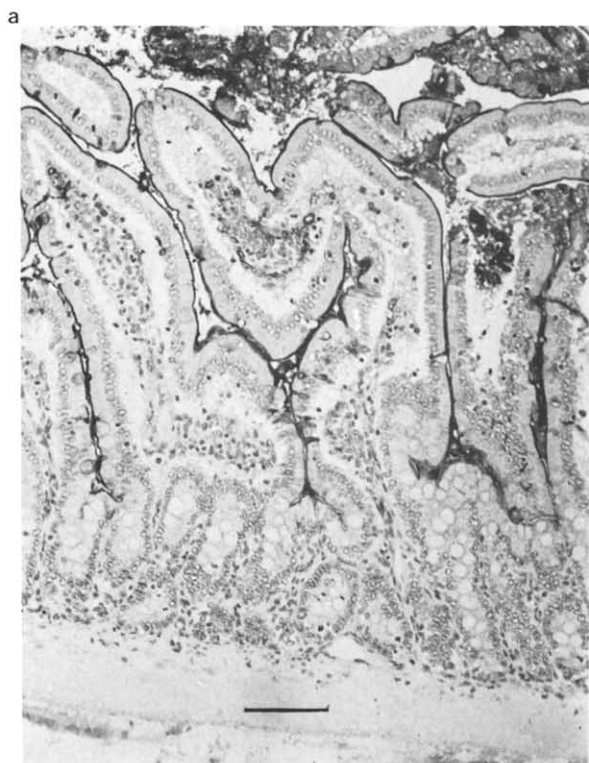
^{125}I -lectin, ^{125}I -BSA (assumed to be a fully digestible protein), or inorganic Na^{125}I , and both urine and faeces were collected during the following 48 h. Results given in table 2 showed that, as expected, virtually all the radioactivity was recovered in the urine when Na^{125}I -BSA was administered, but 21% of the radioactivity was detected in the faeces when rats were given ^{125}I -labelled tomato lectin.

Traces of radioactivity were found in all internal organs examined, even at zero time, and the amount of radioactivity in each increased with time. The most radioactive organ was the liver, but when allowance was made for the relative sizes of the organs, only the kidneys appeared to have significantly more radioactivity than the others (table 1). In all organs examined, except the liver, most (>80%) of the radioactivity at zero time was trichloroacetic acid-precipitable, but by 3 h only a third or less of the radioactivity was not soluble in trichloroacetic acid. As the distant organs received their supply of radioactivity via the bloodstream, the proportion of radioactivity soluble in trichloroacetic acid presumably reflected that in the circulation. In a subsequent experiment, blood samples were taken into heparin and immediately separated into cells and plasma. At zero time, most of the radioactivity was recovered in the cellular fraction, was largely trichloroacetic acid-precipitable and could be recovered from the red blood cell pellet by washing with oligomers of *N*-acetylglucosamine. After 3 h, however, over 60% of the radioactivity was associated with the plasma, of which only 5% was precipitated with trichloroacetic acid.

3.3 Lectin binding to gut wall

The gut wall of rats fed the tomato extract diet was examined histologically. The villus structure appeared normal, with no pathological changes apparent. Sections were stained for tomato lectin using an immunoperoxidase technique: specific

Fig 2 Immunoperoxidase staining for tomato lectin in sections of rat duodenum. Rats were fed on diets containing either 5% casein and 5% tomato juice protein, or 10% casein, as a protein source. Segments of duodenum were fixed, sectioned and stained for tomato lectin as described in section 2. (a) Tomato diet gut with specific antiserum, (b) tomato diet gut with pre-immune serum, (c) casein diet gut with specific antiserum, (d) casein diet gut with pre-immune serum. Scale bar, 100 μm . A similar, but less intense, pattern of staining was obtained with sections prepared from the ileum.



staining was present along with villi of both the duodenum (fig.2) and the ileum, and also appeared to be present within capillaries. Electron microscopy revealed marked specific staining on microvilli, and confirmed the presence of tomato lectin in capillary vessels (not shown). This observation is of major interest because the highly toxic lectin PHA binds to the glycocalyx of the villi (and subsequently there is flattening and disruption of the villus structure) [8], whereas the non-toxic lectin from the garden pea (*Pisum sativum*) does not bind to the mucosal surface of the gut [16]. Our findings are the first demonstration that the binding of a lectin to intestinal villi is not harmful per se, and need not necessarily lead to villus degeneration.

3.4 Human experiments

To assess whether the fate of tomato lectin in the human gut might parallel that observed in our rat experiments, one of us (D C K) consumed 10 μ Ci 125 I-labelled tomato lectin in 10 ml fresh tomato juice containing 1 g BSA, after overnight fasting. Blood samples were drawn immediately before lectin consumption and after 15, 30 min, 1, 2, 3 and 6 h, followed by immediate separation into blood cells and plasma. Most (typically 70% or more) of the blood radioactivity was recovered from the plasma, reached a plateau after 30 min and was mainly trichloroacetic acid-soluble (only 18% on average could be precipitated with trichloroacetic acid). The cell-bound radioactivity, however, was trichloroacetic acid-precipitable and could be removed by washing with oligomers of *N*-acetylglucosamine. By assuming a blood volume of 5.5 l, we calculate that at the plateau level, the circulating radioactivity was 2.3% of that administered, 0.6% cell-bound and 1.7% associated with the plasma.

The uptake of 125 I into the thyroid was also measured using an Elscint Whole Body Scanner. After 4 h the thyroid uptake was 1% of that administered, and since an uptake of about 20% would be expected for inorganic Na^{125}I in that time, a crude estimate of lectin digested would be 5%. The thyroid uptake after 48 h was 12%, and since the uptake of free Na^{125}I in that time would have been around 50%, the proportion of radioactivity entering the circulation could be estimated at 24% of that ingested. Despite the inevitable

crudeness of the calculations, it is clear that the tomato lectin was not fully digested.

3.5 Conclusions

In conclusion, it appears that the non-toxic lectin from tomato fruits resists digestion in the gut of the rat or the human. In rats, the lectin binds to the intestinal villi without causing their flattening or disruption, and it seems likely that the lectin would behave in a similar way in the human gut. An unrelated lectin of similar specificity, WGA, has been shown to bind to intestinal villi *in vitro* [17], and could probably do so *in vivo*, since traces of biologically active WGA have been recovered from human faeces after oral consumption of a large dose of wheat germ [18]. Unlike wheat germ, however, tomato fruits are commonly consumed as part of a normal diet, and relatively large doses of lectin may sometimes be ingested. Since tomato lectin can reach the intestine in a biologically active form, it could exert significant biological effects.

The tomato lectin is known to inhibit lymphocyte transformation induced by recall antigens or allogeneic cells *in vitro* [12,19], and might therefore have immunosuppressive properties *in vivo*. If so, parenteral administration or oral consumption of the lectin might benefit patients with auto-immune disorders, or impair the immune resistance of patients with some malignant diseases. Furthermore, its presence in the diet might influence the pathogenesis of coeliac disease, since the latter is associated with the binding of gluten to poorly differentiated saccharide structures within the gut of susceptible individuals [20], and a non-toxic lectin might modify the development of clinical symptoms by competitive binding to villi. We feel the properties of tomato lectin justify further investigation on its potential actions *in vivo*.

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