

Invited Review

The induction of gut hyperplasia by phytohaemagglutinin in the diet and limitation of tumour growth

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Summary. The growth of a transplantable murine non-Hodgkin lymphoma tumour, developing either intraperitoneally as an ascites tumour or subcutaneously as a solid tumour, has been shown to be markedly diminished by including phytohaemagglutinin (PHA), a lectin present in raw kidney bean (*Phaseolus vulgaris*) in the diet. In NMRI mice fed PHA within the range 0.45-7.0 mg/g diet, tumours which developed during a 10 day period after subcutaneous injection of cells were about 35% of the dry weight of those in lactalbumin-fed (control) animals. The reduced rate of growth occurred in a dose-dependent manner within the range 0.45-3.5 mg/g diet. Based on these observations it has been suggested that a competition between the gut epithelium undergoing hyperplasia and the developing tumour may occur for nutrients from a common body pool, and this may be an important factor with regard to the observed initial low level of tumour growth following the feeding of a PHA-containing diet. Observations which showed that the level of hyperplasia of the small bowel in response to feeding the PHA diets was higher in non-injected mice compared to those which had been injected with tumour cells substantiated the concept of competition between gut and tumour for nutrients etc. required for growth. Experiments with a second murine tumour cell line (a plasmacytoma) in Balb/c mice gave similar results indicating that the effect of PHA was not restricted to a single tumour system.

Key words: Phytohaemagglutinin, Lectin, Hyperplasia, Gut growth, non-Hodgkin lymphoma, Tumour growth, Polyamines

Introduction

It is well known that the supplementation of semi-synthetic diets with the lectin phytohaemagglutinin (PHA), present in raw kidney bean (*Phaseolus vulgaris*), causes a fully reversible, dose-dependent hyperplastic growth of the small intestine in rats (de Oliveira et al., 1988; Bardocz et al., 1990, 1995; Pusztai, 1991). The lectin binds extremely efficiently to the brush border epithelium of the digestive tract and is partially endocytosed into the circulation (Pusztai et al., 1988, 1992; Pusztai, 1991; Bardocz et al., 1995). In rats it has been shown that high doses of PHA (0.2-0.8 g/kg body weight per day) cause damage to the intestinal mucosa, result in an overgrowth of coliform bacteria in the gut lumen (Pusztai et al., 1993) and significantly reduce the fractional rate of protein synthesis in skeletal muscle (Palmer et al., 1987; Bardocz et al., 1992). The plasma insulin level was depressed by a PHA dose of 0.02 g/kg body weight, which represented conditions causing minimal depression of growth and muscle atrophy (Bardocz et al., 1996). Plasma glucose levels, however, remained stable over the whole PHA dose range. A major loss of body lipids has been observed both in rats (Grant et al., 1987; Bardocz et al., 1996) and mice (Bardocz et al., 1994a,b). It is also evident that PHA affects the immune system since a powerful humoral anti-lectin IgG-antibody response has been shown to occur after inclusion of the lectin in the diet (Pusztai et al., 1992).

Putrescine, spermidine and spermine (collectively referred to as polyamines) are known to be present in all mammalian cells and that they are molecules of importance in a series of cellular processes which are vital in the control of cell growth (Tabor and Tabor, 1984; Pegg, 1986; Heby and Persson, 1990; Bardocz, 1993). Polyamines stabilise the conformation of nucleic

acids and are involved in the stabilisation of cellular membranes (Marton and Morris, 1987). They are also implicated in the modulation of receptors and ion channels (Seiler et al., 1996). A large number of studies have provided clear evidence that polyamines are important in mechanisms involved in the development and growth of tumours (Tabor and Tabor, 1984; Pegg, 1986; Bachrach and Heimer, 1988; Heby and Persson, 1990; Hessels et al., 1991; Sarhan et al., 1991, 1992; Bardocz, 1993; Pryme et al., 1995). Polyamines are also known to play major roles in plant growth and development (Tiburcio et al., 1993; Borrell, et al., 1997; Walden et al., 1997).

The stimulatory effect of PHA results in an extensive absorption of amino acids and energy-producing molecules from the gut lumen (Bardocz, 1989; Bardocz et al., 1990). There occurs simultaneously a major transport of polyamines from the blood circulation into the intestinal mucosa and a sequestration of large amounts of polyamines in the stimulated tissue is thus observed before signs of cell proliferation and hyperplasia become evident (de Oliveira et al., 1988; Bardocz et al., 1990, 1995; Pusztai, 1991).

An initial event in cell proliferation is the induction of the enzyme ornithine decarboxylase (ODC), the first enzyme in the polyamine biosynthetic pathway (Pegg, 1986; Johnson et al., 1989; D'Agostino et al., 1990), and this occurs before both the onset of nucleic acid and protein synthesis. Koninkx et al. (1996) have investigated the effect of PHA isolectin E4 on polyamine concentrations and ODC activity in proliferating and differentiating Caco-2 cells. It was demonstrated that polyamine levels in control cells were highest during the early phase of proliferative cell growth and lowest in the stationary phase. Incubation with PHA resulted in a significant increase in polyamine content during the late proliferative phase of growth. ODC activity was high during intensive proliferation and growth, but was lower when proliferation slowed down or ceased entirely. During differentiation Koninkx et al. (1996) were able to show that as the ODC activity fell close to zero, polyamine levels also decreased. The importance of ODC in cancer cells, because of its involvement in the *de novo* synthesis of polyamines, prompted much research directed towards the development of selective inhibitors of the enzyme. The production of difluoromethylornithine (DFMO), a specific inhibitor of ODC activity (Bey et al., 1987), arose great hopes with respect to cancer treatment since it was shown to be extremely effective in reducing the growth of transformed cells *in vitro*. Unfortunately, however, clinical trials proved to be extremely disappointing in that DFMO had only a limited effect on tumour growth (Schechter et al., 1987; Sunkara et al., 1987) and failed to inhibit the compensatory growth of organs (Danzin and Mamont, 1987). In addition, under conditions where *de novo* polyamine synthesis is repressed, it has become apparent that organs and tumours can compensate for this by increasing the level of uptake of polyamines from the

blood. This process consists of a carrier-mediated, energy-dependent mechanism and many types of cells appear to have a single transporter for the uptake of polyamine molecules (Seiler and Dezeure, 1990).

The fact that DFMO has only had limited success has been attributed to three factors: 1) if ODC is inhibited then the tumour is able to obtain sufficient amounts of polyamines from exogenous sources in order to sustain growth, the diet, being a major source of polyamines (Bardocz, 1993), would provide a continuous supply, 2) colonic bacteria represent a potential source of polyamines, and 3) polyamines may be re-utilized extremely effectively following their release from dead cells. An anti-cancer strategy based only on inhibition of polyamine synthesis is thus almost certain to fail due to the following reasons: 1) polyamines are present in essentially all foodstuffs, and at present polyamine-free or even restricted diets are not readily available, dietary control of polyamine intake is at present not feasible, 2) sterilization of the gut (e.g. with antibiotics) is in practice impossible such that contribution of polyamines to the body pool from bacterial sources cannot be avoided, and finally, 3) it is not possible to interfere with the re-utilization of polyamines already existing in the body. Based on these factors we have adopted a novel approach to manipulate the levels of polyamines in the body using the special properties of PHA, which, as mentioned above, causes an extensive accumulation of polyamines in the gut tissues, the aim being to restrict the availability of these molecules for tumour cells. The induced hyperplastic growth of the small bowel was expected to effectively function as a competitor for the growing tumour by reducing the body pool of polyamines, and in doing so reduce the rate of tumour growth. The gut mucosa appears to be one of the most active tissues in the body, illustrated by the fact that an estimated 40% of the total protein synthesising activity in the body per day occurs in this organ (Bardocz et al., 1990). This is presumably further increased during a period of PHA-induced hyperplasia where the demand for extraneous polyamines must increase significantly. This review concerns recent work on the use of PHA as a dietary supplement to induce hyperplastic growth of the gut and reduce tumour growth in mice.

PHA in the diet, gut hyperplasia and effect on other organs

Bardocz et al. (1994a,b) have shown that the growth and metabolism of the digestive tract of mice was affected by PHA. It was observed that although the wet weight of the stomach decreased that of the small intestine, caecum and colon significantly increased. Most of the changes were still seen after the tissues had been lyophilised. PHA affected the entire digestive system of mice in a similar manner to that observed in rats, inducing a particularly large increase in the weight of the small intestine (Table 1). It is almost certain that

Dietary PHA limits tumour growth

the effect in mice is identical to that studied in detail in rats and it is thus likely that it is induced by the same mechanism. The increased turnover of the epithelial cells leads to a speeding up of the loss and degradation of villus cells in the lumen. The released lectin is then free to react with the epithelium in more distal parts of the gut, and the physiological stimulus of PHA therefore in due course affects the entire alimentary canal.

The increase in content of protein, RNA and DNA in a 20 cm section of the jejunum of mice given PHA in the diet further confirmed that the lectin was a growth factor for the small intestine (Bardocz et al., 1994a). Furthermore, the structure of the jejunum changed considerably and its morphology became characteristic for a rapidly proliferating tissue. The length of the crypts in PHA-fed animals showed marked elongation due to the hyperplastic response, whereas the villi only showed slight signs of elongation (Fig. 1).

Using immunocytochemical visualisation in order to

Table 1. Effect of diet on the relative sizes of individual organs.

	Organ dry weight (mg)	
	La diet	PHA diet
Stomach	75.6±8.4	66.0±5.2
Small intestine	334.4±54.2	580.6±130.3
Large intestine	142.3±28.3	123.2±33.6
Caecum	38.0±11.7	46.2±19.1
Colon	101.3±21.7	77.0±21.1
Liver	560.6±85.2	447.0±80.3
Kidneys	126.8±22.2	96.0±14.6
Lungs	64.6±14.2	55.6± 7.1
Heart	45.2±3.7	35.4±4.5

One group of mice was fed La diet and a second group PHA diet (7 mg/g). Mice in both groups (5 animals/group) were injected s.c. with 2×10^6 Krebs II cells. After 12 days mice were sacrificed, various organs dissected out and immediately frozen in liquid nitrogen. After lyophilisation of the tissue the dry weight of the individual organs was determined. The results are expressed as \pm SEM for 5 individuals/group.

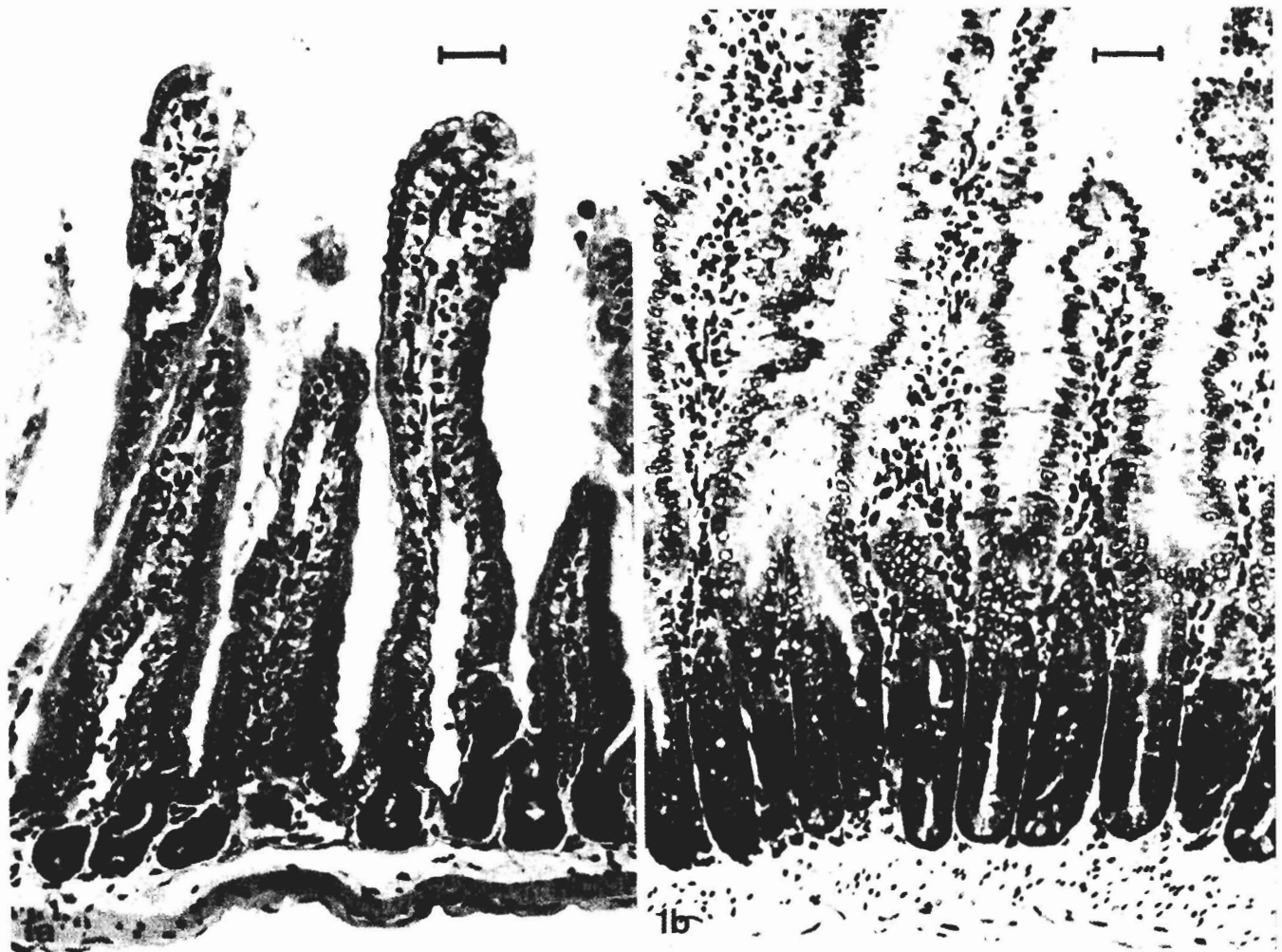


Fig. 1. a. Jejunum from control-fed (La) mice, stained with haematoxylin and eosin. b. Jejunum from PHA-fed (7 mg/g diet) mice, stained with haematoxylin and eosin. The villi are slightly elongated but the crypts show marked elongation due to the PHA-induced hyperplasia. Bar: 40 μ m.

show the localisation of PHA in the mouse jejunum, intense binding of the lectin to the microvillous border of the villi was observed and also evidence of endocytosis was noted (Fig. 2a). Techniques of freeze substitution and resin embedding on sections of rat jejunum permitted visualisation of PHA in the hyperplastic crypt microvillous border and demonstrated the occurrence of endocytosis in the crypt epithelium (Fig. 2b).

Since the gut is the first line of defence in the body, keeping the structure of the gut intact is crucial for the survival of the species. If the demand for energy and essential molecules to support the PHA-induced growth of the gut cannot be satisfied by the diet, then the body will inevitably draw on its own reserves in order to be able to support compensatory gut growth.

PHA in the diet and development of Krebs II tumours

In dietary experiments female NMRI mice either received a semi-synthetic control diet based on 10% lactalbumin (La) as the protein source or diets where a required amount of the lactalbumin was replaced by an appropriate amount of kidney bean (*Phaseolus vulgaris*) protein to provide diets containing the required PHA content (for details see Pryme et al., 1996a). In most experiments the kidney bean diet contained 7mg of lectin/g diet, and based on a daily food intake of 6g/day, the diet provided approximately 42mg PHA/mouse/day. Female NMRI mice were injected either intraperitoneally (i.p.) or subcutaneously (s.c.) with 2.0×10^6 Krebs II non-Hodgkin lymphoma tumour cells in order to induce the formation of ascites or solid tumours respectively (Pryme et al., 1994a).

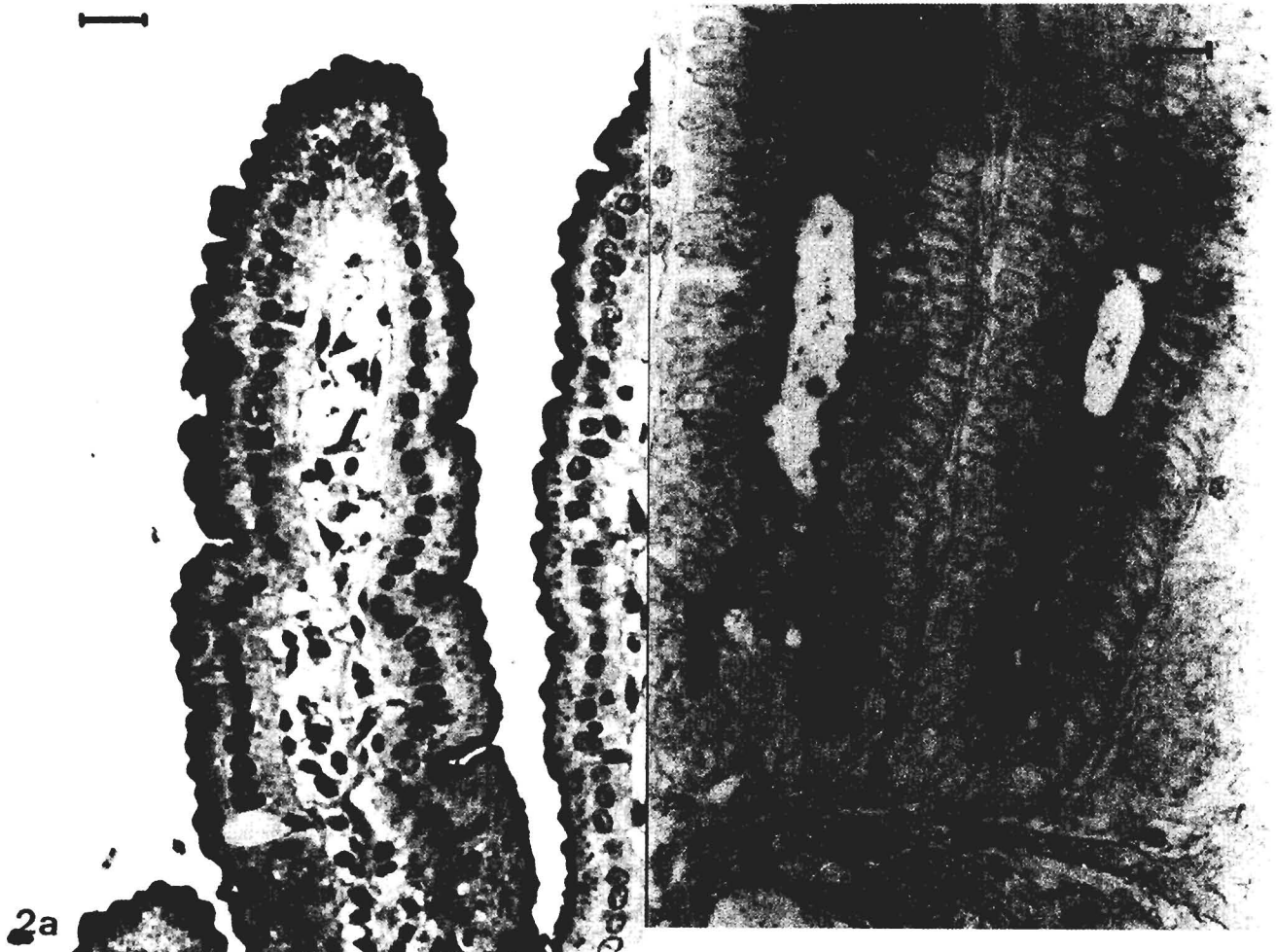


Fig. 2. a. Immunocytochemical localisation of PHA in the mouse jejunum. There is intense binding of the lectin to the microvillous border of the villi and evidence of endocytosis. b. Immunocytochemical localisation of PHA in the rat jejunum. Freeze substitution and resin embedding permits localisation of PHA in hyperplastic crypt microvillous border and demonstrates the presence of endocytosis in the crypt epithelium. Bar: 20 μ m.

Dietary PHA limits tumour growth

In an initial experiment an increase in body weight was observed in mice fed La diet between days 2 and 4 after i.p. injection of Krebs II tumour cells which was indicative of the formation of ascites tumours. Mice on the PHA diet, however, showed a progressive decrease in body weight during the first 7 day period of observation amounting to an individual loss of about 16% of body mass (Pryme et al., 1994a). Although the mice exposed to the PHA diet exhibited a fall in body weight, attributed to a loss of body lipids (Bardocz et al., 1994a,b; Pryme et al., 1996b) the growth of the tumour cells was not totally abolished. Fig. 3 shows that after 5 days the total number of tumour cells present in the peritoneal fluid aspirated from mice fed on the PHA diet, was 53% of the corresponding number in La fed individuals. At 9 and 12 days the figures were 41 and 63% respectively. Mice injected s.c. with Krebs II tumour cells and fed on the control La diet showed an increase in body weight after 3 days indicating the commencement of growth of solid tumours. Individuals on the PHA diet, however, exhibited an increase in weight only 7-8 days following the injection of cells. From these observations it was apparent, therefore, that feeding mice PHA diet results in a slower initial rate of proliferation of Krebs II cells growing either intraperitoneally as an ascites tumour or subcutaneously as a solid tumour.

When rates of cell production in ascites tumours were calculated as a "growth factor" during the course of

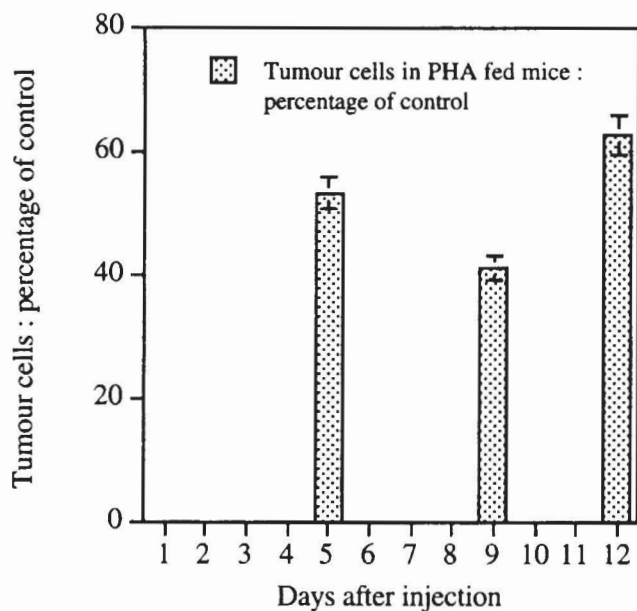


Fig. 3. Two groups of mice were injected i.p. with 2×10^6 Krebs II cells and then one group was fed on a La (control) diet and the other on PHA. On days 5, 9 and 12 three mice were sacrificed in each group and the number of cells in the ascites tumour determined using a hemocytometer chamber. For each time-point the number of cells in tumours in PHA fed mice is expressed as a percentage of those in La fed mice (\pm SD).

three time periods following i.p. injection of cells it was evident that the rate of proliferation of cells in the mice fed PHA diet was much slower during the middle of the growth period (days 5-8) than that in the La fed mice (Pryme et al., 1994b). During the later phase of growth (days 9-12) a higher rate of Krebs II cell growth was observed in PHA compared to La fed animals. An explanation for these results may be the accompanying hyperplastic growth of the gut which occurs in parallel with tumour growth. The lower level of growth early after injection could be due to the PHA-induced accumulation of polyamines in the mucosal tissue of the small intestine (Table 2). The sequestration of polyamines in the gut mucosa thus reducing the extraneous supply to the tumour. It can be seen that during a period of 12 days of feeding a PHA-containing diet, large increases in the content of putrescine, spermidine and spermine occurred in the tissue. Total polyamines in the control (La fed) animals amounted to 552.1 nmol/mg DNA in comparison to a value of 905.6 in PHA fed mice, representing a 64% increase. In the presence of a growing tumour the corresponding value, however, was reduced to 795 nmol/mg DNA, which was about 88% of the control. This would indicate that the growing tumour reduced the polyamine content of the small intestinal tissue, possibly suggesting a competition for polyamines between the PHA-induced hyperplastic growth of the gut and the proliferating tumour cells. Interestingly, in La fed mice with developing ascites tumours, the intestinal mucosa showed a decreased putrescine content (-27.2%) but increased content of both spermidine (+8.4%) and spermine (+42.7%), indicating that the growing tumour modulated polyamine levels in the small intestine. It thus seems likely that an initial slow rate of propagation of Krebs II cells growing as an ascites tumour in PHA-fed mice can be attributed to a decreased availability of extraneous polyamines necessary to support tumour growth. Moreover, the results suggest that the mucosa of the small intestine is more readily able to obtain polyamines from extraneous sources than the tumour. Once the hyperplasia of the gut has reached its limit, however, then the body pool of polyamines would

Table 2. Putrescine, spermidine and spermine content of the mucosa of the small intestine (nmol/mg DNA).

	La	PHA	La + tum	PHA + tum
Putrescine	86.4 \pm 25.9	261.1 \pm 78.3	62.9 \pm 18.9	195.0 \pm 58.5
Spermidine	252.1 \pm 75.6	357.8 \pm 107.3	273.3 \pm 82.0	327.1 \pm 98.1
Spermine	213.6 \pm 64.1	286.7 \pm 86.0	304.8 \pm 91.4	272.9 \pm 81.9

Two groups of mice (5 animals/group) were fed La diet and two groups PHA diet (7 mg/g). Mice in one of each La and PHA groups were injected i.p. with 2×10^6 Krebs II cells (La + tum, PHA + tum). The other two groups served as non-injected controls. After 12 days mice were sacrificed, the small intestine dissected out, washed and immediately frozen in liquid nitrogen. After lyophilisation of the tissue the DNA content was measured and the content of putrescine, spermidine and spermine determined (for details see Bardocz et al., 1994b). The results are expressed as \pm SEM for 5 individuals/group.

Dietary PHA limits tumour growth

recover thus enabling the rate of tumour cell proliferation to accelerate (Pryme et al., 1994b).

In an experiment where two groups of mice were followed for a period of 14 days following s.c. injection it was observed that only one individual out of five survived the whole period when fed the control diet while four out of five survived when maintained on the PHA diet. In another experiment the PHA diet was shown to increase the survival time of NMRI mice by a factor of 2-3 following i.p. injection of Krebs II non-Hodgkin lymphoma tumour cells (Pryme et al., 1996c). These experiments thus illustrated the fact that supplementation of the diet with the lectin derived from *Phaseolus vulgaris* prolonged the survival time of tumour-bearing mice.

In order to test whether or not the effect of feeding a PHA diet on tumour growth was restricted to the Krebs II non-Hodgkin lymphoma cell line in NMRI mice, a second tumour line (a plasmacytoma, MPC-11) was tested in a second strain of mice (Balb/c). Ten days after subcutaneous injection of MPC-11 cells, plasmacytoma tumours which developed in female Balb/c mice fed on a diet containing PHA weighed only about 38% of those fed an La control diet (Pryme et al., 1996d). Pre-feeding with the lectin caused a further 50% reduction in tumour weight. In contrast to the reduction in tumour size the inclusion of PHA in the diet elevated the mean dry weight of the small intestine in a dose-dependent manner. The results showed that gut hyperplasia was able to occur even in the presence of the developing tumour. A lipolytic effect of PHA was demonstrated at high concentration. In other experiments with the MPC-11 cell line the dry weight of the total intestine in mice pre-fed with 7.0 mg PHA/g diet prior to subcutaneous injection of tumour cells gave a value which was 53.2 mg above that observed when the animals were fed the same diet commencing on the day of injection. This was in contrast to the corresponding value for the tumour where a value 58 mg lower was observed. Thus the gain in dry weight of the gut caused by the hyperplastic response virtually matched the observed reduction in tumour weight (Pryme et al., 1997a). It is evident, therefore, that the effect of PHA is not merely restricted to one type of tumour in a single, specific strain of mice.

In a dose-response experiment NMRI mice were injected s.c. with Krebs II cells and fed on La diet or diets containing the kidney bean lectin PHA within the range 0.45-7.0 mg/g diet (Fig. 4). The tumours which developed during a 10 day period were less than 50% of the dry weight of tumours in control La fed mice (Pryme et al., 1996a). In non-injected mice fed the same diets gut hyperplasia occurred in a dose-dependent manner with a maximal effect between 1.75-3.0 mg. In mice developing tumours the maximal hyperplasia occurring in response to PHA was shifted to the highest concentration. A lipolytic effect of PHA was observed above 1.75 mg in control mice and the highest concentration had a major effect on body weight. The lower concentrations of lectin in mice injected with

tumour cells appeared to partially protect against loss of body fat and body weight. Since in these experiments the index of hyperplasia did not correlate with tumour size, the results suggested that other factors, in addition to polyamines, which have an initial role in hyperplastic growth, are important in slowing down tumour progression (Pryme et al., 1996a).

Effect of switching between PHA and La diets on tumour development

In an initial experiment concerning the importance of the timing of feeding mice a PHA-containing diet, three groups of mice were injected i.p. with Krebs II cells, and of these two were fed La diet while the third group received PHA (7 mg/g). On day 4 after injection one of the La fed groups was switched to PHA for 4 days before the animals in all three groups were sacrificed. In mice fed PHA for 8 days the number of cells recovered in the ascites tumour was 50% relative to the control (La) group diet while in the group switched to PHA after 4 days the corresponding value was about 60% (Pryme et al., 1994a). It was thus evident that it was possible to effectively slow down the growth of tumour cells by switching from La to PHA diet even though the tumour was already established in the host.

Further dietary switch experiments were performed in which mice were pre-fed on either La or PHA diets for 3 days prior to the injection of tumour cells and then the animals kept on the same diets or they were switched after s.c. injection of Krebs II cells (Pryme et al., 1996b). The results clearly demonstrated the effectiveness of PHA in reducing tumour growth. The lowest tumour weight recorded was observed in mice after a total of 11 days on the PHA diet, including a 3 day pre-feeding period with the lectin. A marked reduction was also observed in animals pre-fed for 3 days with PHA before tumour cells were injected and the diet was switched to

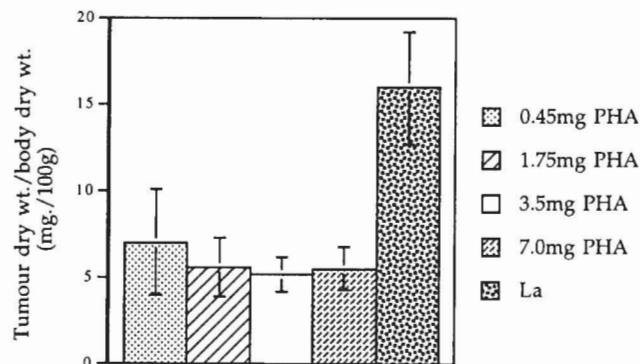


Fig. 4. Five groups of mice (five per group) were injected s.c. with 2×10^6 Krebs II cells. One group was fed La (control) diet and the other four PHA-containing diets within the range 0.45-7.0 mg/g diet. After 10 days all mice were sacrificed and the tumours excised. Tumours were frozen immediately in liquid nitrogen before being lyophilised for dry weight determination. Tumour size is expressed as tumour dry weight/dry body weight (mg/100g) (\pm SD).

La immediately after injection, perhaps indicating some form of prophylactic effect of the lectin. A switch of diet from standard pellet diet to PHA on the day of injection also caused a large decrease in tumour weight (Pryme et al., 1994a).

Since it had been observed that PHA induced a hyperplastic growth of the gastrointestinal tract within a 12 day period of feeding a PHA diet (Bardocz et al., 1994a) it was important to study the effects of dietary switch on the resulting hyperplasia of the gut. Based on proportional dry weight values of the small intestine (percentage dry weight related to dry body weight) it was evident that despite the presence of the developing tumour PHA was able to induce hyperplasia in all the three groups of animals which were fed the lectin during either part or for the whole duration of the experimental period (Pryme et al., 1996b). Although there was a marked degree of hyperplasia of the small intestine in mice pre-fed for 3 days before injection of tumour cells and then fed on PHA for a further 8 days (dry organ weight: 363.4 ± 34.6 mg), the final weight was 38% lower than that in non-injected PHA fed mice (580.6 ± 130.3 mg). In comparison in non-injected La-fed mice the small intestine weighed 334.4 ± 54.2 mg. The induction of hyperplasia by PHA had therefore apparently prevented the loss in small bowel weight which was observed during tumour development. This was in contrast to the La fed, tumour-bearing mice where the small intestine weighed 229.0 ± 28.2 mg, i.e. a loss of 105.4 mg, equivalent to a reduction of 31.5% in dry weight. These results add further support to the existence of a major competition between the developing tumour and PHA-induced gut hyperplasia for nutrients and growth factors. Other experiments showed a clear tendency between a reduction in tumour weight and an increase in the dry weight of the small intestine (Pryme et al., 1996e).

Polyamines in cells and organs

Bardocz et al. (1997) have shown that the intracellular concentrations of putrescine, spermidine and spermine were higher by a factor of approximately three in tumour cells obtained from mice fed for 8 days on PHA diet after injection of tumour cells, compared to those only fed La. These observations together with others (Bardocz et al., 1994a; Pryme et al., 1995, 1996f) clearly indicated that feeding mice a PHA-containing diet resulted in a modulation of intracellular polyamine levels and, furthermore, the changes appeared to be correlated with the level of proliferative activity of the tumour cells.

Following mitosis the cellular polyamines are redistributed between the two daughter cells. These post-mitotic cells then have to accumulate spermidine and spermine during the G1 phase before they can enter S-phase (Seiler et al., 1996). Cells which have a short cell cycle, such as Krebs II cells, will therefore have a great demand for polyamines. The required intracellular

concentration of polyamines in cancer cells is achieved by a combination of *de novo* synthesis and a high uptake rate from extraneous sources (Seiler et al., 1996). If the extraneous source of polyamines is restricted then it would be expected that this would have a marked effect on tumour cell growth. Since it is known that the PHA-stimulated hyperplasia of the gut mucosa in mice causes a sequestration of polyamines in the tissue (Bardocz et al., 1994a,b; Pryme et al., 1996a) then one would expect that this "normal" growth activity would compete with a developing tumour for polyamines from a common body pool. In this context it has been shown that the total polyamine content in the mucosa of the small bowel in mice injected i.p. with Krebs II tumour cells and fed PHA for 12 days was approximately 30% lower than that observed in non-injected animals (Bardocz et al., 1994a). In La fed mice the comparative value was 13%.

The results described above indicate a relationship between the demand made by the gut for exogenous polyamines and limited tumour growth. It would appear that the build up of polyamines observed in cells from tumours in mice fed for 8 days on the PHA diet is related to cell cycle events, i.e. a large proportion of cells are presumed to be in G1/S phase. A stabilisation of the hyperplastic growth of the gut will reduce the demand for extraneous polyamines. An increased proportion of tumour cells will be able to obtain the additional polyamines they require from the body pool in order to promote entry into S phase and later into mitosis. Bardocz et al. (1997) demonstrated that at least a threefold increase in polyamine content had to occur in Krebs II cells before they were able to perform active growth. These results would suggest, therefore, that the Krebs II cells alone are unable to produce sufficient amounts of polyamines themselves by *de novo* synthesis through the ornithine decarboxylase system to support growth and proliferation. These observations add further support to the concept that a continuous supply of extraneous polyamines is essential in order to sustain tumour growth. If the supply becomes limited, for example by PHA-induced hyperplasia of the gut, then the level of proliferation of the tumour can be reduced.

Pryme et al. (1997b) have described results which suggested that in response to a developing tumour some organs may be induced to increase their production of polyamines, these may be exported and transported in the blood to the tumour in order to satisfy the requirements of the growing tumour for an increased extraneous supply. This implies that polyamines synthesised *de novo* by the tumour, and those provided by the diet (possibly also a contribution from colonic bacteria, although this has not been confirmed) are together inadequate to support neoplastic growth.

Conclusions and future perspectives

It has become apparent that inclusion of the plant lectin PHA in the diet results in a reduced rate of growth of two different types of tumour cells in two separate

strains of mice. The results suggest a correlation between dietary PHA and the induction of a rapid series of events occurring in the host leading to a reduction in growth of a non-Hodgkin lymphoma and a plasmacytoma. Altered polyamine content and weight of tissues indicates that interorgan competition between the tumour and the gut can be used to manipulate metabolism in tumour-bearing mice. The promising results obtained hitherto with PHA indicate that lectins, with such properties as those exhibited by the kidney bean (*Phaseolus vulgaris*) lectin, may prove to be of extreme value when designing new approaches of anti-cancer strategy, especially if combined with the use of a polyamine-free/depleted diet.

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Dietary PHA limits tumour growth

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