# SOME PATHOLOGICAL ASPECTS OF RAW LIMA BEAN (*Phaseolus lunatus* Linn. FEEDING TO GROWING RATS

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## ABSTRACT

Some pathological changes accompanying raw lima bean (RLB) feeding to growing rats were investigated. Among the gross pathological observations were severe pancreatic hypertrophy and spleen atrophy. The relative weight of the liver was significantly ($P < 0.05$) decreased while the kidney weight was significantly ($P < 0.05$) increased. The relative weight of the heart was unaffected. At necropsy, the small intestine of rats fed 40% RLB and above were dilated and filled with light-yellow gassy liquid.

Histopathologically, livers of rats fed 40% RLB and above showed focuses of coagulative necrosis and marked congestion of hepatic sinusoids and centrolobular veins. The spleen showed marked depletion of lymphoid follicles and medullary sinusoids. There was evidence of marked congestion of renal blood vessels in the high RLB diets. No lesions were observed in the heart tissues.

The morphology of the intestinal apical microvilli were generally disrupted. They appeared stunted and fragmented especially in the rats fed 40% RLB and above. Mononuclei cell infilterate characterized the lamina propria of the small and large intestinal microvilli. No microvilli abnormalities were detected in rats fed 100% cooked lima bean (control) diet.

## INTRODUCTION

It has long been recognized that the nutritive value and protein digestibility of edible species of some common legumes are very poor unless subjected to cooking or some other form of heat treatment (Liener and Kakade, 1969). Other reports (Beko, *et al.*, 1972; Brambila *et al.* 1978; Liener, 1976; King *et al.* 1980) have shown that growing rats and chicken fed diets containing raw field bean *(Vicia faba* L.), soya bean *(Glycine max)* and kidney bean *(Phaseolus vulgaris)* as the major source of protein, suffered significant growth retardation, pancreatic hypertrophy and even death if the ingestion was prolonged. These deleterious effects have generally been attributed to the presence of a number of chemical substances such as the trypsin inhibitors, phytohaemagglutinins (lectins), cyanogenic glycosides, globulins, saponins and tannis (Warsy and Stein, 1973; Marguardt *et al.*, 1976; Eggum, 1980; Liener, 1980). Inspite of the numerous investigations, the actual mode of action of most of these toxic factors still remains somewhat conjectural.

However, recent studies in our laboratory (Ologhobo and Fetuga,

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1983; Aletor and Fetuga, 1984 a & b) demonstrated that at least part of the growth depressing effect of raw lima bean (RLB) feeding to the rat is mediated via deranged digestion and/or absorption of some dietary nutrients as well as marked alteration in the normal activities of several hepatic and extra-hepatic enzymes.

The present paper examines some pathological aspects of RLB intoxication of growing rats in cooked lima bean based diets.

## MATERIALS AND METHODS

*Management of experimental animals*

Sixty weanling rats (43 – 45g) of the Wistar strain obtained from the pre-clinical rat coloney of the College of Medicine, University of Ibadan were used in this study. All rats were divided into six groups of ten rats each (5 males + 5 females) to give identical mean group weights (44.11 – 44.50g). The rats were thereafter housed in stainless steel individual metabolic cages.

*Diets:*

There were six diets in all. The composition (%) of the basal diets are given in earlier studies (Aletor and Fetuga, 1984 a & b). It was formulated such that cooked lima bean furnished all the 16% crude protein level on dry matter basis. This served as the control. The other diets (2–6) were derived from the basal by replacing 20, 40, 60, 80 and 100% of the cooked lima bean with the raw bean. All diets were hand mixed starting with the smaller ingredients to ensure uniform blending. Water and food were available freely for the 21-day experimental period.

*Pathological studies*

At the end of the experiment, all rats were stunned and killed by neck decapitation with a surgical knife. Their livers, pancreases, kidneys, spleens and hearts were quickly dissected out, freed of any adhering fat, blotted free of blood and the weights recorded. Pieces of tissues were also immediately removed from the duodenum, jejunum, large intestine and the caecum. All tissues were preserved in 10% buffered formol saline.

Sections of the liver, pancreas, kidney, spleen and heart from each rat were fixed in Bouin's fixative, trimmed, embedded in paraffin, sectioned at 5 – 6μ and stained with haematoxylin and eosin (H & E). Intestinal, samples were similarly prepared except that sections were cut at 1μ thick and stained with toluidine blue (King *et al.* 1980).

*Statistical analysis:*

Data on the relative organ weights were subjected to factorial analysis of variance and regression using the IBM–computer SPSSH (1976 version).

## RESULTS

*Relative organ weights:*

The curves with their respective prediction equations showing the effects of RLB on the relative weights of some of the organs are shown in figures I and II. Pancreas weight was very significantly ($P < 0.001$) increased while spleen weight was significantly ($P < 0.001$) decreased by RLB. There was significant ($P < 0.05$) decrease in liver weight while kidney weight was

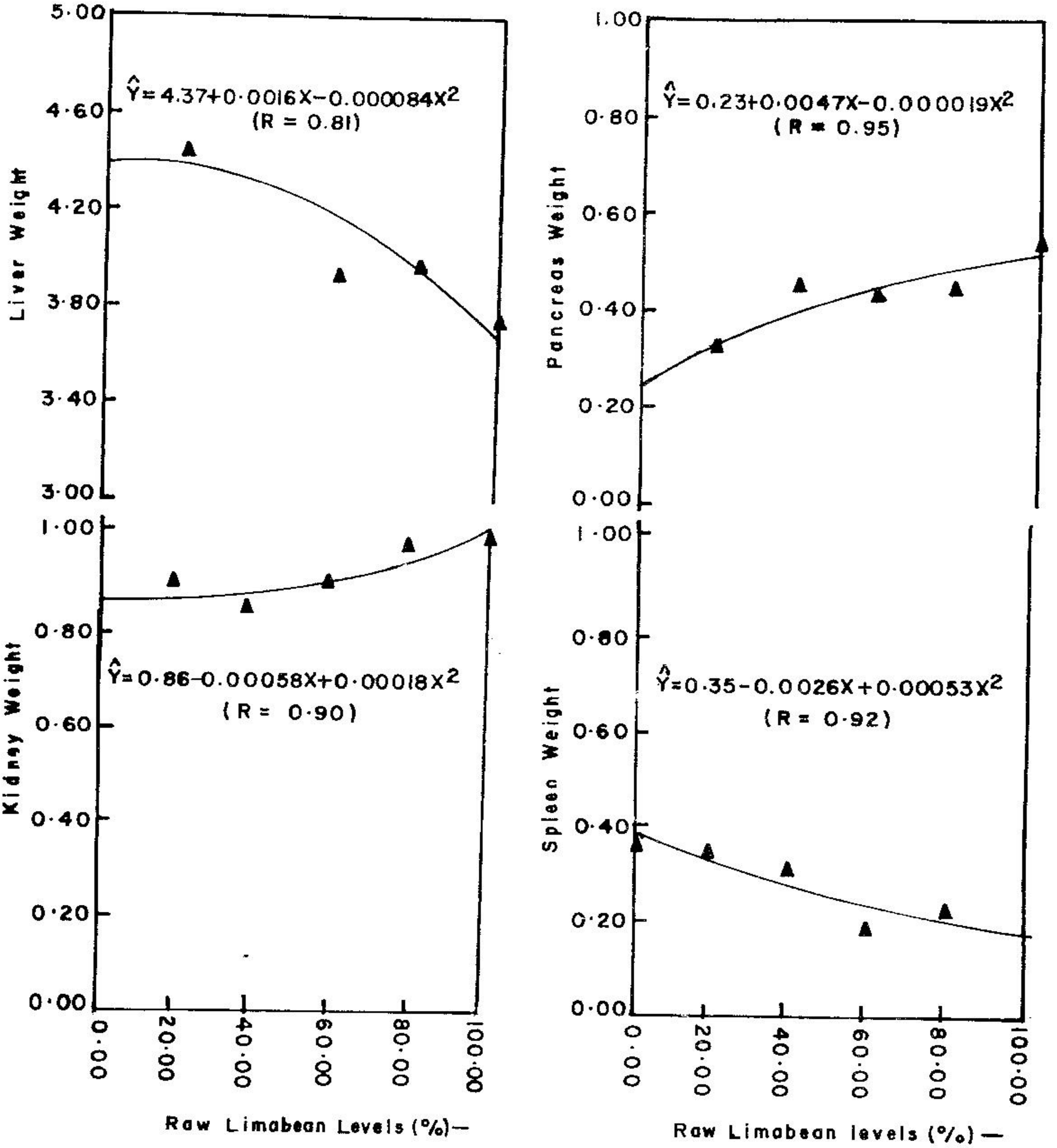


*Fig. 1. Relative Organic Weights (g/100g Body Wt.) As Affected by Raw Limauean Levels.*

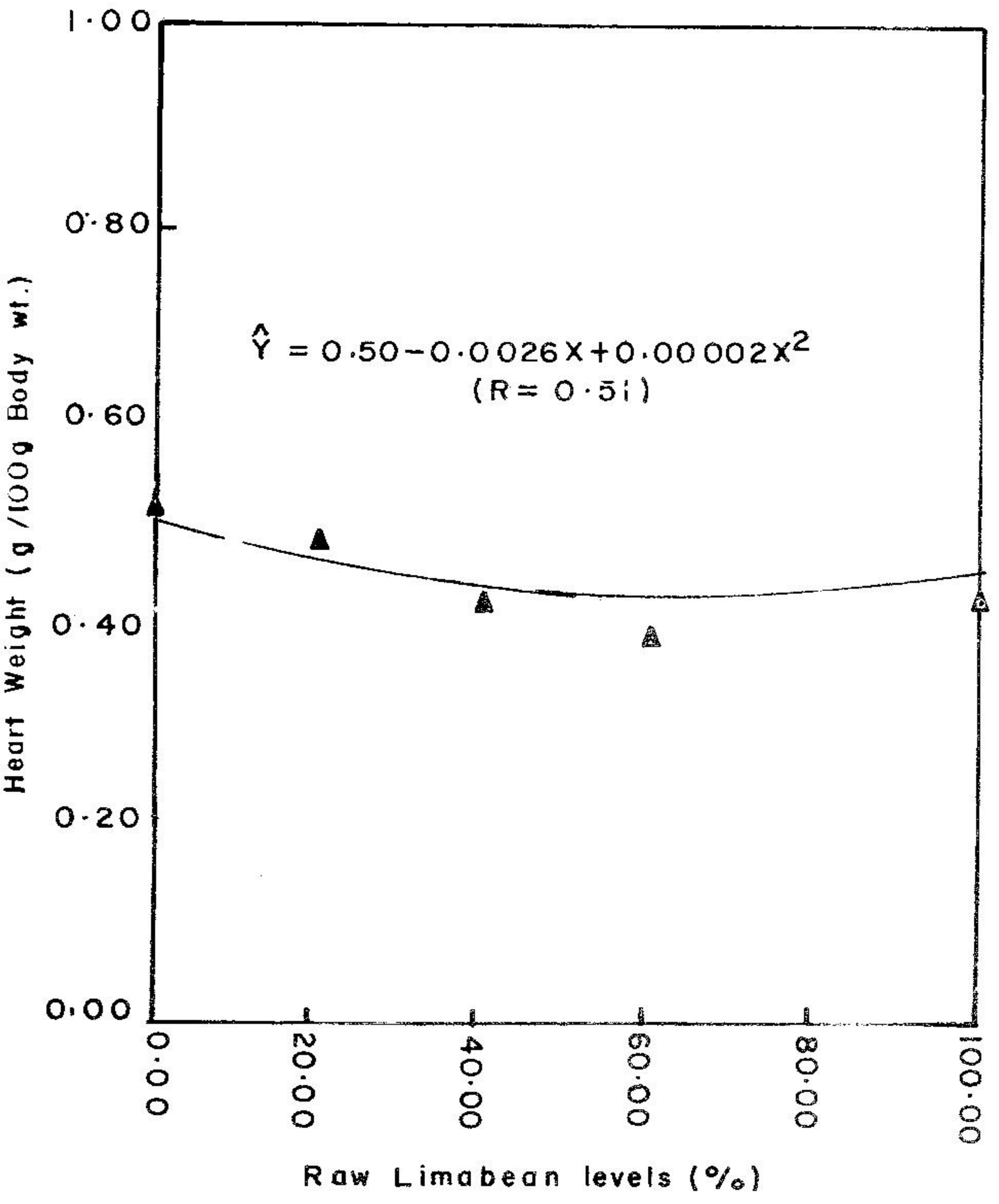


*Fig. 2. Relative weight of Heart as Affected by Dietary Raw Limabean leaves.*

significantly ($P < 0.05$) increased. The heart weight was relatively unaffected by RLB. The relative weights of the liver, pancreas, spleen and kidney were significantly ($P < 0.01$) correlated with RLB levels.

*Gross pathology*

At the end of the experiment, all rats fed 60% RLB and above were moribund. At necropsy, the small intestine of the rats fed 40% RLB and above were dilated and filled with light-yellow gassy liquid. The walls of the small intestine and caecum generally appeared thin By far, the most consistent pathological manifestations of dietary RLB were severe pancreatic hypertrophy and spleen atrophy The liver was also slightly atrophied.

*Histopathology*

Distinct from those fed on an all-cooked lima bean (control) diet, the livers of the rats fed RLB showed focuses of coagulative necrosis. There was also congestion of hepatic sinusoids and the centrolobular veins especially in rats consuming over 40% RLB (Plate 1).

Pancreatic arcinar hypertrophy was a consistent feature at all levels of RLB. There was also marked congestion of renal blood vessels and capillaries. The spleen of rats at all levels of dietary RLB showed marked depletion of the lymphoid follicles and medullary sinusoids (Plate 2). No cardiac lesions were observed.

There were increasing degrees of disruption of the normally uniformly arranged apical micro-villi of the duodenum and jejunum with increasing levels of RLB. Lesions in the intestinal epithelium were usually more pronounced in both the duodenal and jejunal regions than in the large intestine in rats fed 40% RLB and above. In comparison with rats fed cooked lima bean (control diet), the microvilli of the RLB–fed rats generally appeared stunted and fragmented (Plate 3). The lamina propria of the small and large intestine were characterized by eosinophils and lymphocytes. Floculent debris and mucus were quite often associated with disrupted microvilli especially in the large intestine and caecum.

## DISCUSSION

The present report is corroborative of earlier ones (Beko *et al.,* 1972; King *et al.,* 1980) that the ingestion of raw or improperly cooked soyabean (*Glycine max*), field bean (*Vicia faba*) and kidney bean (*Phaseolus vulgaris*) elicit certain physio-pathological changes which could result in death. In this regard, information on limabean (*Phaseolus lunatus*) whose protein productivity potential is surpassed only by soyabean (Luse, 1975), remains comparatively scanty. The high potency of the toxic chemical substances within RLB is indicated by the pancreatic hypertrophy and spleen atrophy observed even at levels of 20 – 40% replacement of the cooked bean with the raw. The absence of such pathological conditions in the cooked lima bean fed rats, suggests a susceptibility of the causative chemical substances to thermal inactivation; while the significant correlations between the relative weights of the liver, pancreas, spleen, kidney and the RLB levels (figure 1), emphasizes the importance of dose levels of these chemical substances in the observance of these changes.

*Plate 1: Photomicrograpn of the rat's liver. Left: Normal liver (A): Right: Liver showing congestion of the centrolobular vein and periportal necrosis (B). H & E stain. X 25.*

*Plate 2: Photomicrograph of the rat's spleen. Left: Normal spleen (C). Right: Spleen showing depletion of the white pulp (D). H & E stain. X 25.*

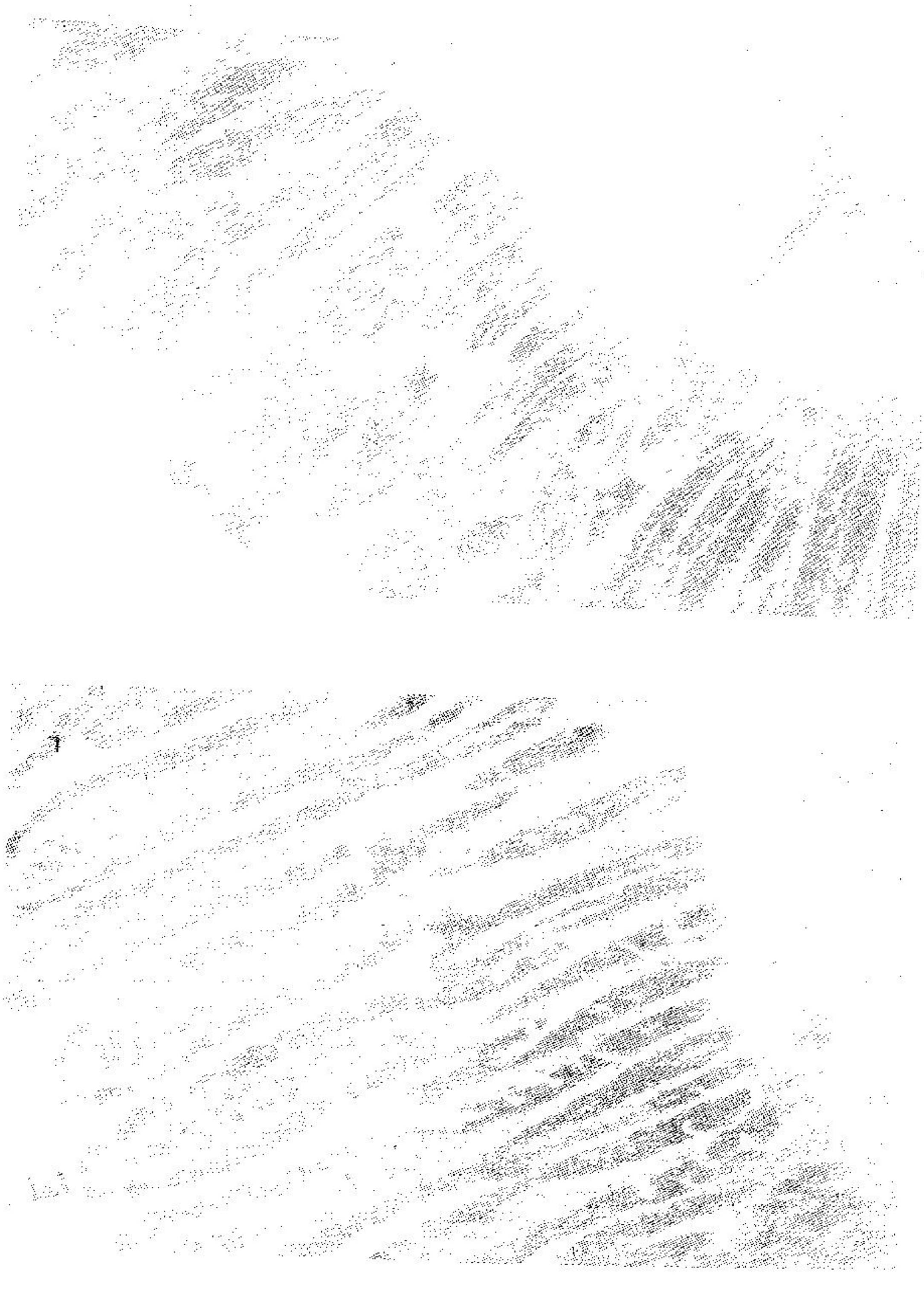

*Plate 3: Photomicrograph of the small intestine. Left: Normal jejunal villi (E) Right: Jejunum showing fragmented and denuded villi (F). Toluidine blue stain. X 16.*

Although the mechanisms of pancreatic hypertrophy still remains largely unclear, Khayambashi and Lyman (1966) reported that rat pancreas which had been perfused with blood plasma from a rat fed trypsin inhibitor secreted increased amounts of enzymes. They concluded that trypsin inhibitor might be liberating a hormone-like agent which enlarges pancreatic acinar and hence the overall secretory function.

Pathological lesions in animals injected intraperitoneally (i.p) with kidney and castor bean extracts containing lectin have been described by Szper-Seyfriedowa (1964). Parenchymatous and fatty degeneration of some tissues were reported. Focal necrosis and fatty changes have been observed (Sharon and Lis, 1972; Ologhobo and Fetuga, 1983) in the liver, while the kidneys and myocards showed distention of capillary vessels with numerous thrombi following acute i.p. toxicity of certain bean agglutinins (lectins). The close similarity of these findings with our present study does not only implicate the lectin component of lima bean but also implies a high potency even in it's native form.

The marked depletion of the lymphoid follicles and medullary sinusoids of the spleen may have been contributory to the over 50% decrease in the relative weight of the spleen. This study lends support to our previous ones (Ologhobo and Fetuga, 1983; Aletor and Fetuga, 1984b) in which we proposed that the elevation of the activity of certain hepatic and serum transaminases following the feeding of RLB and i.p. injection of lima bean and cowpea lectin, may result from the death of certain hepatic cells as well as a loss in certain permeability characteristics.

Liener (1976) has opined that the lectins are important contributory factors to the poor nutritive value of some legumes particularly those belonging to the genus, *Phaseolus*. In this regard, they are believed to combine with cells lining the intestinal wall, thus causing a non-specific interference with the absorption of all nutrients. These findings coupled with the present morphological evidence suggest that some degree of malabsorption in the intenstine occurs following RLB feeding. Additionally, a loss in the permeability of the intestinal epithelium is suggested since rats feed high RLB diets accumulated very watery, yellowish liquid in the lumen.

The eosinophils which characterized the lamina propria of the small and large intestine in the present study are known to contain profribrinolysin (Junqueira, 1975) which is believed to play a role in keeping the blood liquid especially when its fluidity is altered by pathological processes. However, whether the accumulation of the eosinophils was a response to the presence of lima bean lectin per se is being investigated.

Therefore, if the full nutritional potential of lima bean is to be realised, intensive efforts must be applied towards the incorporation of desirable biochemical and nutritional characteristics into breeding programmes which uptill now have tended to emphasize yield, pest and/or disease resistance. The development of new genetic stock (through selection) with minimum levels of some or all of these genetic stock (through selection) with minimum levels of some or all of these

lima bean based toxic substances, is highly desirable in view of the distressing consequences of feeding RLB to rats, and the risk of similar toxicological effects in animals of agricultural importance.

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