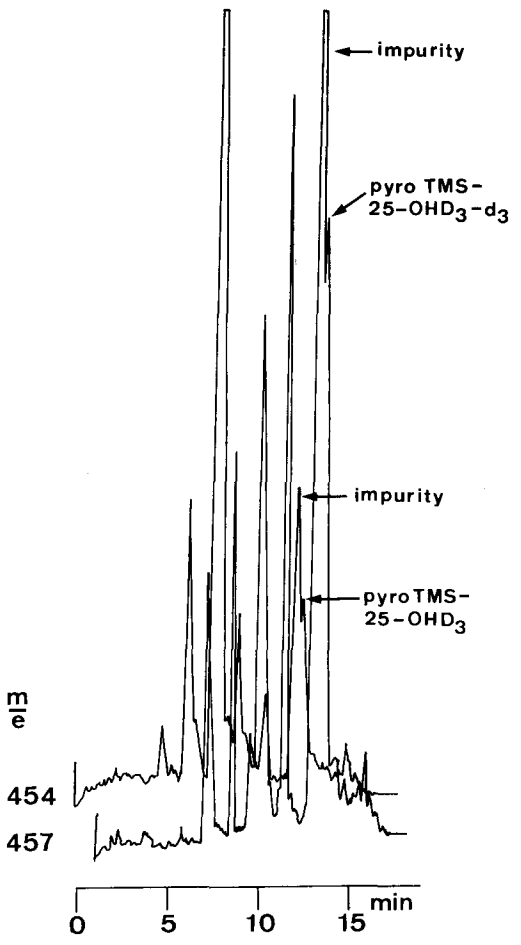

Fig. 3. GC-SIM chromatogram of silylated serum sample; fraction: 25-hydroxycholecalciferol. The sample was purified by column- and thin layer chromatography on alumina. Details in figure 1.

after the addition of cholecalciferol and 25-hydroxycholecalciferol to serum samples. Similar assay for 1,25-dihydroxycholecalciferol in serum is under investigation.

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The reported occurrence of L-canavanine in soya bean, *Glycine max*[1]

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Summary. The reported occurrence of L-canavanine in soya bean, *Glycine max* could not be verified by enzyme treatment of the extracted non-protein amino acids of the seed.

Recently, Fischer et al.[3] reported the putative occurrence of L-canavanine in the seed of the soya bean, *Glycine max*. This secondary plant metabolite is a principal nitrogen-storing non-protein amino acid of certain legumes[4]. Fischer et al.[3] made the interesting observation that when ground meat was adulterated with soya bean meal, automated amino acid analysis of the meat sample extract produced a ninhydrin-positive peak believed to be canavanine. This finding, if confirmed, would be important since canavanine is a highly toxic non-protein amino acid and soya bean serves as an increasingly important source of human dietary protein. Moreover, canavanine possesses potent insecticidal properties[4], and, if present, could be utilized in experimental studies of toxic natural product-herbivore interaction. Several extensive plant surveys have established that canavanine's distribution is limited to the Papilionoideae (Faboideae), a major subfamily of the Leguminosae[5-7], where it has provided insight into the phylogenetic relationship of certain legumes[8,9]. The efforts of Tschiersch[7], Bell et al.[9], and Lackey[10] all failed to produce positive evidence for seed canavanine in *G. max*. Several ppm of canavanine have been reported in the garden onion, *Allium cepa*[11] and in the fungus of commerce, *Agaricus campestris*[12]. However, these reports have either been challenged[13] or have not been corroborated by independent methods for establishing and verifying minute levels of canavanine. In each instance, canavanine's purported occurrence was predicated solely upon isolation of a ninhydrin-positive substance having the column retention time of canavanine.

Materials and methods. The soya bean sample consisted of an approximately equal mixture of the following varieties: Woodworth, Forrest, Bonus, Dare, York, Williams, Corsoy, Essex Hood 75, and Mack. The soya bean meal (350 g), prepared as described previously[14], was extracted,

processed, and subjected to ion-exchange chromatography by a procedure developed for isolating canavanine from defatted jack bean seeds[15]. The purified soya bean extract (10 ml) was brought to pH 2 with HCl and stored at −35 °C. Throughout the entire purification procedure, the various experimental conditions were selected to minimize canavanine loss by cyclization to deaminocanavanine[16].

The production of L-canaline and urea, formed by hydrolytic cleavage of L-canavanine in the soya bean extract, was evaluated by incubating 1 ml of the purified soya bean extract with 4 mg of commercially prepared arginase (57 units/mg), 1 mM $MnCl_2$, and 50 mM tricine buffer (pH 7.6) in a final vol. of 2 ml. After 2 h at 37 °C, the reaction mixture was treated with 2 ml of cold, 10% (w/v) trichloroacetic acid, centrifuged, and the supernatant solution (1 ml) evaluated for urea by a colorimetric assay that could detect 10 nmoles of urea[17]. A colorimetric assay for canaline of comparable sensitivity is lacking. However, canaline can be carbamylated to O-ureido-L-homoserine and then assayed by a colorimetric procedure capable of detecting 10 nmoles of the latter compound[18]. This was attempted by reacting 10 mM carbamoyl phosphate and a large excess of jack bean ornithine carbamoyltransferase with the above reaction mixture.

Results and discussion. Defatted soya bean meal contains a ninhydrin-positive substance (less than 10 μmoles/100 g) that eluted with the column retention time of canavanine (282 min) and can be isolated from an ethanolic extract of the seed meal by ion-exchange chromatography[15]. The isolated soya bean amino acid reacts with pentacyanoammonioferrate (PCAF), under neutral conditions, to produce an orange-red colored complex; in contrast, canavanine formed a vivid, magenta-colored complex. Arginase treatment of the soya bean PCAF-responsive material failed to produce detectable urea and canaline, the reaction products, as determined by colorimetric analysis. In addition, arginase did not affect significantly the subsequent absorbance of the amino acid-PCAF complex. (Comparable experiments, conducted with 2 mM L-canavanine yielded 98.8% of the anticipated urea.) Thus, while soya bean produced a ninhydrin- and PCAF-positive amino acid which might be valuable in assessing the presence of soya bean additives in other foodstuffs, this marker amino acid was not canavanine. Once again, this study amply demonstrated the inadvisability of relying upon the elution position of a ninhydrin-positive substance as the sole criterion for establishing the presence of an amino acid in a biological material.

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Essential fatty acids (EFA) deficiency and liver mitochondria

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Summary. 2 dietary fats, namely, hydrogenated coconut oil and safflower seed oil were fed at 20% levels to weanling male albino rats for a period of 2 months after which the animals were sacrificed and oxidative phosphorylation measured in liver mitochondria. This ratio was more in the unsaturated-fat-fed group of rats compared to the saturated-fed ones for glutamate and malate; in the case of succinate no such change was noticed.

As a part of our program to effectively understand the various parameters in lipid metabolism as influenced by dietary fats, we reported recently the cholesterol esterification activities in intestines and pancreas[1] and the regulation of hepatic lipogenic enzymes[2] as a function of dietary fats. Lipids have been demonstrated to be essential for mitochondrial functions and it is also known that mitochondria are the most sensitive indicators of EFA depletion. The present study represents our findings on the effect of 2 dietary fats on the mitochondrial oxidation of some of the TCA cycle intermediates.

Materials and methods. 20 weanling male albino rats were divided into 2 groups of 10 each and fed a diet containing either hydrogenated coconut oil or safflower seed oil. The diet consisted of fat-free casein (20%), fat (20%), cane sugar (10%), cornstarch (45%) and vitamin-mineral mixture (5%). The feeding experiment was carried out for a period of 2 months after which the animals were sacrificed by decapitation. Liver was removed immediately and homogenized (10%) in isotonic solution (sucrose 0.25 M + $CaCl_2$ 1.8 mM) using a Potter-Elvejhem homogenizer and filtered through muslin cloth. Nuclei were removed at 700 × g and the mitochondria obtained at 10,000 × g. This was washed once and used in our experiments. The amount of mitochondria used in each experiment consisted of the mitochondrial suspension in sucrose solution corresponding to approximately 200 mg of fresh liver and was put in the main compartment of the 2 side-arm Warburg flask. Ox-