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-

Effect of dietary fats on oxidative phosphorylation

Substrate	Group I (hydrogenated coconut oil)			Group II (safflower oil)		
	P_i (μmoles)	O (μatom)	P/O	P_i (μmoles)	O (μatom)	P/O
Glutamate	12.3	6.5	1.9	15.2	4.75	3.2
Malate	7.1	4.14	1.7	10.4	3.85	2.7
Succinate	11.5	6.9	1.6	11.0	6.87	1.6

The reaction mixture for the oxidative phosphorylation study contained[5]: phosphate buffer, pH 7.4, 50 μmoles, $MgCl_2$ 10, ATP 6, NaF 40, sodium glutamate 30 or malate 40 or succinate 40, cytochrome c, 0.03 mg, hexokinase 2 mg, glucose 10 mg, mitochondrial suspension 1.0 ml corresponding to approximately 200 mg fresh liver and 0.25 M sucrose solution to make up the volume to 3.0 ml.

ygen uptake was measured at 37 °C every 5 min for 30 min. Phosphate esterification was determined by the disappearance of P_i in presence of glucose-hexokinase trapping system. At the termination of the experiment (30 min) the flasks were quickly transferred to an ice-bath and the reaction stopped immediately by adding 2 ml of 20% ice-cold TCA. The precipitated proteins were removed by centrifugation and the phosphorous content of the protein-free filtrate was determined by the method of Fiske and Subbarow[3]. Initial phosphorous level was determined in control flasks in which the protein was precipitated after equilibration. The net phosphate uptake was calculated from the difference between the inorganic phosphate contents of the various experimental flasks and the corresponding zero time flask. The oxygen uptake during the same time interval was calculated from the manometric readings.

Results and discussion. The P/O ratios, calculated as moles of phosphorous consumed/atoms of oxygen taken up, was depended upon the type of the dietary fat (table). The P/O ratios were 1.9, 1.7 and 1.6 for glutamate, malate and succinate respectively in group I compared to 3.2, 2.7 and 1.6 in group II. These values showed that except when succinate was used as the substrate, the unsaturated fat-fed rat liver mitochondria exhibited higher P/O ratios compared to the saturated coconut oil group; in the case of the succinate no change was noticed. The data also pointed out that the hydrogenated coconut oil fed rats esterified less phosphorus during the oxidation of the same substrates as did the other group. These indicated that the lack of essential fatty acids (EFA) results in a decreased esterification of the high-energy phosphate the oxidation of some of the TCA cycle intermediates. The hydrogenated coconut oil rat liver mitochondria oxidized the substrates at slightly faster rates than did the liver mitochondria of safflower oil fed rats.

A nutritional deficiency of essential fatty acids in the rat results in a structurally altered liver mitochondria[4]. Since EFA enters the structures of the membranes, it would be expected that in EFA-deficiency structural changes would take place in these membranes. Sufficient data are there to show that the uncoupling in 'deficient' mitochondria results from a relatively induced structural changes in the latter[4,6,7]. Some studies suggested that the mitochondria are the most sensitive indicators of EFA depletion. An involvement of oxidative processes with unsaturated fatty acid-deficiency has been shown by Kunkell and Williams[9] who reported an increased cytochrome oxidase activity in livers from fat-deficient rats without affecting the succinoxidase activity. Alfin-Slater and Aftergood[10] pointed out that EFA-deficient mitochondria had an increased tendency to swell, were more fragile and had a lower P/O ratio than normal hepatic mitochondria indicating an uncoupling of oxidative phosphorylation and interference with the normal membrane structure. The differences observed in the P/O ratios during the oxidation of α-ketoglutarate, β-hydroxybutyrate, glutamate or malate on one hand, and succinate on the other as reported by Klein and Johnson[11] suggested that the uncoupling might be restricted to the DPN-linked portion of the electron transport system.

The normal rat livers in the present study represent the livers of rats on safflower seed oil and the EFA-deficient ones represent those on the hydrogenated coconut oil. In the present study also, no difference was noticed in the P/O ratio when succinate was used as the substrate. This supports the view that the uncoupling of oxidative phosphorylation or any defect of the electron transport system is at or near the level of NADH oxidation. Our results with the supportive evidence of the previous workers suggest that the structural changes associated with the electron transport flow gives rise to changes in the phosphorylation efficiency in the EFA-deficient liver mitochondria. These probably mean that the change in phosphorylation potential of the liver mitochondria is due to the differences in the mitochondrial lipids especially phospholipids which reflect the nature of the dietary fat. It is conceivable that due to the changes in the fatty acids, phospholipids in the 'deficient' mitochondria provide an environment unfavorable to the synthesis of high energy intermediates of oxidative phosphorylation. Indeed, it has been proposed[12,13] that the uncoupling of the oxidative phosphorylation may be due to a molecular defect caused by the absence of EFA in the structure. In view of the interrelationship between the structural changes in the mitochondria and electron transport and oxidative phosphorylation, it may be that an EFA-deficiency results in structurally defective mitochondria and that normal mitochondrial phospholipid fatty acid pattern is an essential requisite for the normal oxidative phosphorylation process. The differences in the mitochondrial lipogenic enzyme levels as observed by us[2] may be a follow-up of the changes in the phosphorylation potentials as noted in this paper.

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