Increase in Na^+,K^+-ATPase enzyme units in liver and kidneys from essential fatty acid deficient rats

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Summary. (^{3}H)-Ouabain binding to liver and kidney preparations was utilized to estimate the number of Na^+,K^+-ATPase enzyme units in livers and kidneys from rats fed 2% corn oil supplemented or fat-free diets. The specific (^{3}H)-ouabain binding in liver and kidney preparations from fatty acid deficient rats was increased approximately 40%, but the affinity of the binding sites for ouabain (K_d-value) remained unchanged. The increased concentration of Na^+,K^+-ATPase enzyme units observed in the essential fatty acid deficient rats may contribute to the reduced body fat accumulation and elevated heat production observed in these animals.

Na^+,K^+-ATPase is the enzymatic equivalent of the Na pump[2]; operation of this pump has been suggested to be one of the major energy consuming processes in mammals[11]. There is a paucity of data on physiological factors which control Na^+,K^+-ATPase; however, the importance of membrane phospholipids has received recent attention[3]. The fatty acid composition of membrane phospholipids can be altered by varying the type or amount of fat consumed. Mice fed an essential fatty acid deficient diet exhibit an increased specific activity of Na^+,K^+-ATPase in brain homogenates and in isolated synaptosomal membranes[4]. Changes in Na^+,K^+-ATPase activity of Ehrlich ascites tumor cells produced by alteration of membrane fatty acid composition have also been reported[5].

It has been known for some time that rates of energy utilization are elevated in rats fed essential fatty acid deficient diets[6], but the mechanisms involved have not been elucidated. An increase in Na^+,K^+-ATPase activity could contribute to the elevation of cellular thermogenesis in these rats. In this communication, we evaluated the extent of body energy (fat) accumulation in rats pair-fed fat-free or essential fatty acid adequate diets as an indicator of the magnitude to which essential fatty acid deficiency influences dietary energy utilization. Rates of heat production were also determined in a direct calorimeter. The concentration of Na^+,K^+-ATPase enzyme units in liver and kidney from the essential fatty acid deficient and adequate rats was assayed from the concentration of high affinity (^{3}H)-ouabain binding sites in the tissues[7].

Materials and methods. 19-day-old male Sprague-Dawley rats (from Spartan Research Animals, Inc., Haslett, Michigan) weighing approximately 50 g initially, were housed in metal cages having raised wire floors. The room lights were on from 07.00 h to 19.00 h daily. Water was available ad libitum. The rats were fed a fat-free diet for 7 weeks; at which time all the rats had scaly tails and reduced rates of growth. The rats were then randomly divided into 3 groups (8 per group); one group was killed to determine initial body fat content. The remaining rats were pair-fed either a 2% corn oil supplemented diet or a fat-free diet for 13 weeks. The composition of the fat-free diet is described elsewhere[8]. The 2% corn oil diet was formulated by replacing glucose with corn oil on an equal energy basis.

At the end of the experiment, heat production of each rat was measured directly in a gradient layer calorimeter[9]. Rats were then killed and the vena cava was immediately incised. After perfusing the rat with 20 ml of cold saline solution via the left ventricle of the heart to remove blood, the liver and both kidneys were quickly removed. Particulate fractions from these tissues were prepared as described[7] and were assayed for (^{3}H)-ouabain binding[7]. Body fat content of the rats was determined gravimetrically after chloroform : methanol (3 : 2 v/v) extraction.

Results and discussion. The 2 groups of rats consumed equal amounts of energy each day (intake averaged 85 kcal per day), but the b.wt gain of the rats fed the 2% corn oil supplemented diet was greater than that of the rats fed the fat-free diet (table 1). By the end of the experimental

Table 1. Final b.wt, b.wt gain, final carcass fat, carcass fat gain and heat production of rats fed 2% CO or EFAD diets*

	Diet 2% CO	EFAD
Final b.wt (g)	472 ±8	373 ±6**
B.wt gain (g)	162 ±4	64 ±6**
Final carcass fat (%)	11.4±0.7	8.0±0.3**
Carcass fat gain (g)	33 ±3	10 ±1**
Heat production (kcal/h · $kg^{3/4}$)	5.8±0.1	6.5±0.2**

* CO = corn oil supplemented; EFAD = essential fatty acid deficient. Means ± SEM for 8 rats in each group. ** Significantly different at $p < 0.05$.

Table 2. Tissue weights and (^{3}H)-ouabain binding to tissue preparations from 2% CO or EFAD rats*

	Liver Diet 2% CO	EFAD	Kidney Diet 2% CO	EFAD
Weight	13.0 ± 0.4	10.9 ± 0.2**	2.7 ± 0.07	2.7 ± 0.08
Particulate protein (mg/g tissue)	63 ± 3	67 ± 3	61 ± 3	61 ± 2
Specific (^{3}H)-ouabain binding (pmoles/mg protein)	0.10± 0.01	0.14± 0.01**	1.4 ± 0.1	2.0 ± 0.2**
Nonspecific (^{3}H)-ouabain binding (pmoles/mg protein)	1.10± 0.04	1.00± 0.03	1.48± 0.04	1.56± 0.03
K_d-values (μM)	11.6 ± 0.3	12.2 ± 0.3	5.9 ± 0.3	6.0 ± 0.1
(^{3}H)-ouabain binding site concentration (pmoles/mg protein)	3.1 ± 0.3	4.4 ± 0.4**	22 ± 2	32 ± 3**
(pmoles/total tissue)	2511 ±250	3081 ±199	3608 ±315	5339 ±407**

*,** See table 1.

period, rats fed the supplemented diet had gained 2.5 times as much b.wt and 3.3 times as much body fat as the rats consuming equal amounts of energy from the fat-free diet. The essential fatty acid deficient rats produced more heat per unit b.wt than did the rats fed the corn oil supplemented diet. These results are in agreement with an earlier report of increased oxygen consumption[6] in essential fatty acid deficient rats and suggest that essential fatty acid deficient rats have increased cellular thermogenesis.

Rats fed the 2% corn oil supplemented diet had heavier livers than rats fed the fat-free diet, but liver particulate protein content, expressed as mg per g of liver, was unchanged (table 2). Kidney weights and protein content were not influenced by the diets.

The specific (^{3}H)-ouabain binding in liver and kidney preparations from rats fed the fat-free diet was increased approximately 40% (table 2). This increase in (^{3}H)-ouabain binding was specific for saturable binding since nonsaturable binding was not affected by the diet fed. The increase in specific ouabain binding observed in the liver and kidney preparations from essential fatty acid deficient rats was due to an increase in concentration of ouabain binding sites rather than to alterations in affinity for ouabain because the affinity of each binding site for ouabain (K_d-value) remained unchanged. Since the livers of the fat-free group weighed less the specific ouabain binding, expressed on a total liver basis, was not significantly elevated in the rats fed the deficient diet. But specific ouabain binding to kidney preparations from the fat-free group, expressed per total kidney weight, was significantly higher than that of the 2% corn oil supplemented group. The increase in specific ouabain binding observed in the liver and kidney preparations of essential fatty acid deficient rats is consistent with an earlier study[4] in which Na^+,K^+-ATPase activity in brain tissue from essential fatty acid deficient mice was elevated. The mechanism of the dietary induced alteration in ouabain binding remains to be established; however, alterations in membrane lipid composition and/or prostaglandin metabolism may be involved[10].

An increase in membrane associated Na^+,K^+-ATPase enzyme units in essential fatty acid deficient rats may lead to elevated ATP turnover and thus contribute to the lowered energy efficiency observed in these animals. However, it must be emphasized that a change in the number of enzyme units may not reflect enzyme activity in the intact cell. Further studies are needed to evaluate the extent to which alterations in sodium pump activity in vivo, as well as other energy utilizing processes[11], contribute to increased heat production in essential fatty acid deficient rats.

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Isolation of di-N-2-propylpentyl phthalate from human urine[1]

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Summary. A higher percentage of di-N-2 propylpentyl phthalate isolated from the urine of the normals and a lower quantity in the patients suffering from Eales' disease accompanied with the occurrence in patients of another compound which is also most likely a phthalate ester has been correlated with the Eales' disease.

Since its discovery by Henry Eales in 1880[2], a number of unsuccessful attempts have been made by various workers to find the cause of recurrent retinal hemorrhages occurring predominantly in young males[3,4], a condition that has since come to be called as Eales' disease. So far, no definite preventive or curative treatment is available. In our laboratories, we have been attempting to find the cause of this disease, and report our findings.

Ether extracts of urine from the patients and normal individuals were analysed on TLC plates when 2 spots having $R_f \sim 0.9$ and ~ 0.34 were obtained. The concentration of the compounds was diagnostic of the disease, the compound having $R_f \sim 0.34$ occurring in higher concentrations in the patients and the compound with $R_f \sim 0.9$ existing in a higher concentration in the normals (figures 1 and 2). While we have isolated and characterized the product $R_f \sim 0.9$, the compound having $R_f \sim 0.34$ is still under investigation.

Material and Methods. Collection and extraction of urine. Collection of 24-h urine from 27 patients between 20 and 40 years (all males) and 17 normal individuals (all males) of the same age group were made under toluene and were kept refrigerated. All medications were stopped 2 days prior to the collection of urine of the patients under study. Peroxide-free, freshly distilled 30 l of ether was used for the extraction of 15 l of urine each from the patients and normals. The ether extracts on distillation yielded oily coloured products which were fractionated on alumina column for purification.

Chromatographic procedures. TLC plates were run, using benzene methanol (3.5%) mixture, and developed with Follin's reagent[5] in ammonia tank. Purification of the ether extracts was made on alumina column using benzene-methanol (2%).

Results and discussion. The eluates obtained after the column fractionation of the urinary extracts of the normals yielded on evaporation an oil (155 mg, $R_f \sim 0.9$) which was found to be homogeneous on TLC and gas chromatogram. Corresponding eluates from the patients extract yielded