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# Effect of Sodium Chloride, Aspartic Acid and Glutamic Acid on the Oxygen Consumption, Cholesterol and Total Lipids of Liver of Rat

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**Abstract :** Minute changes in pH, NaCl concentration Aspartic acid, Glutamic acid and by inference other acids affect the major pathways from acetyl Co-A. The important differences between experiments with and without buffer show the reversal of results. Glutamic acid increases $O_2$ consumption; cholesterol of liver homogenates in media without buffer decreases $QO_{2,.}$ cholesterol and total lipids in media with rigorously controlled pH at 7.4; similar is the case with aspartic acid.

**Key words :** Sodium Chloride, Aspartic Acid, Glutamic Acid, Oxygen Consumption, Cholesterol, Total Lipids, Liver, Rat

## Introduction

Conjugated linoleic acids (CLA) are a class of positional, geometric, conjugated dienoic isomers of linoleic acid (LA). Dietary CLA supplementation results in a dramatic decrease in body fat mass in mice, but also causes considerable liver steatosis. However, little is known of the molecular mechanisms leading to hepatomegaly (Clement 2002). In liver, the synthesis of cholesterol and fatty acids increases in response to cholesterol deprivation and insulin elevation, respectively. This regulatory mechanism underlies the adaptation to cholesterol synthesis inhibitors (statins) and high calorie diets (insulin). In nonhepatic cells, lipid synthesis is controlled by sterol regulatory element-binding proteins (SREBPs), membrane-bound transcription factors whose active domains are released proteolytically to enter the nucleus and activate genes involved in the synthesis and uptake of cholesterol and fatty acids (Matsuda *et al*, 2001). Aspartic acid and glutamic acid are special in being dicarboxylic and mono-amino acids. Both take part in transamination reactions and are directly related to the Krebs cycle. In convulsive fits in experimental animals and in humans, glutamic acid and asparagines are administered. The living system maintains and work within a narrow pH range, the present work was

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studied with a buffer at a constant pH of 7.4. Work on NaCl, aspartic and glutamic acid is presented here.

## Material and Methods

Slices of fresh liver (central lobe) of albino rat were cut with a blade and placed in 5 ml of Krebs–Ringer phosphate solution. The NaCl concentration of Krebs-Ringer phosphate in various incubating media varied to 7g%, 8g%, 9g%, 10g% an 11g%. The pH of the buffer was maintained in every media by adding minute quantities of 0.1 N NaOH solutions. The experimental sets contained 5 mg of aspartic and glutamic acid.

The results obtained are based upon the average of 54 readings and calculations of $Q_{O_2}$ at $37^0$C and at intervals of 10, 20, 30, 40, 50, and 60 minutes in the Warburg's apparatus. After observing readings for oxygen consumption ($Q_{O_2}$), the slices were dried at a lower temperature of 65ºC, weighed and placed in 5 ml solvent ether for 15 minutes. The ether was transferred to another weighed watch glass and evaporated. The residue was weighed indicating the total lipids. The cholesterol in the residue was estimated by Libermann Burchard reaction.

## Discussion

The observations are presented in Table 1.The most significant inference which can be drawn between the experiments with and without buffer is that the results have been totally reversed. Glutamic acid increases the $O_2$ consumption and cholesterol of the liver homogenate in media without buffer whereas on the other hand it decreases the $O_2$ consumption and cholesterol of the liver homogenate in media with buffer (at 7.4 pH),. Laborit (1966, 1972) has been the first to suggest and use potassium and magnesium salts of Aspartic acid in the treatment of hyperammonieme produced by injections of ammonium chloride and to protect the rats against convulsive fits. Thus the present study also confirms the results obtained by Laborit. According to Laborit, low concentrations of Aspartic acids of tricarboxylic acid that became impossible in an atmosphere of oxygen without $CO_2$ because of the lack of conversion of pyruvic acid into malic

**TABLE 1 : Plus and minus percent effect of NaCl (%) Aspartic acid (Asp) and Glutamic acid (Glu) on Oxygen consumption ($QO_2$), Total lipids (TL) and Cholesterol (Ch) on the liver of rat with buffer 7.4 and without buffer.**

| Treatment | | NaCl | 7g | 8g | 9g | 10g | 11g |
|---|---|---|---|---|---|---|---|
| With buffer at 7.4 | Asp | $Q_{O_2}$ | +36.1 | +39.2 | +46.2 | +18.2 | +12.3 |
| | | TL | +31.0 | +29.0 | +43.4 | +26.0 | +16.8 |
| | | Ch | +26.2 | +25.1 | +39.5 | +23.2 | +12.4 |
| | Glu | $Q_{O_2}$ | –38.2 | -41.2 | –29.8 | –30.2 | –32.1 |
| | | TL | –29.3 | –32.1 | –34.1 | –35.6 | –31.8 |
| | | Ch | –24.0 | –25.3 | –28.2 | –30.1 | –28.5 |
| Without buffer | Asp | $Q_{O_2}$ | –39.9 | –36.6 | –27.4 | –22.6 | –20.4 |
| | | Ch | –34.4 | -24.5 | –21.0 | –12.2 | –18.6 |
| | Glu | $Q_{O_2}$ | +128.2 | +78.5 | +71.2 | +73.4 | +69.4 |
| | | Ch | +13.0 | 7.0 | +53.0 | +18.2 | +14.1 |

or oxaloacetic acid through carboxylation. A certain percentage of $CO_2$ is necessary in the blood and extra cellular fluids. Acidosis can be corrected by injecting chemicals to bring about a change in the pH. Tobin (1972) has also shown the effect of pH on oxidative phosphporylation of rat liver mitochondria. **Matsuda** *et al* (2001) have indicated the need of an activating protein (SCAP) for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. Again in a related study Mustard *et al* (2004) have discussed how dietary linoleic acid increases and palmitic acid decreases liver cholesterol and receptor proteins in young pigs. Udinstev (1970) found stimulation of steroid synthesis *in vitro* and *in vivo* in adrenal glands of man and animals by glutamic acid. Hawk (1976-77) found that these two amino acids glutamic and aspartic acid can influence biosynthesis of cholesterol in liver through various reactions. Glutamic acid at constant pH of 7.4 reduces $O_2$ consumption ($Q_{O_2}$), total lipids and cholesterol of liver

which is associated with its conversion to corticosteroids. Non-esterified long-chain fatty acids (myristic, palmitic, oleic and arachidonic), added at low amounts (around 20 nmol/mg protein) to rat liver mitochondria, energized by respiratory substrates and suspended in isotonic solutions of KCl, NaCl, RbCl or CsCl, adjusted to pH 8.0, induce a large-scale swelling followed by a spontaneous contraction. Such swelling does not occur in alkaline solutions of choline chloride or potassium gluconate or sucrose. These changes in the matrix volume reflect a net uptake, followed by net extrusion of KCl (or another alkali metal chloride) and are characterized by the following features :

(1) Lowering of medium pH from 8.0 to 7.2 results in a disappearance of the swelling-contraction reaction.

(2) The contraction phase disappears when the respiration is blocked by antimycin A.

(3) Quinine, an inhibitor of the K(+)/H(+) antiporter, does not affect swelling but suppresses the contraction phase.

(4) The swelling phase is accompanied by a decrease of the transmembrane potential and an increase of respiration, whereas the contraction is followed by an increase of the membrane potential and a decrease of oxygen uptake.

(5) Nigericin, a catalyst of the K(+)/H(+) exchange, prevents or partly reverses the swelling and partly restores the depressed membrane potential.

These results indicate that long-chain fatty acids activate in liver mitochondria suspended in alkaline saline media the uniporter of monovalent alkali metal cations, the K(+)/H(+) antiporter and the inner membrane anion channel. These effects are presumably related to depletion of mitochondrial Mg(2+), as reported previously [Schonfeld *et al*, 2002], and are responsible for the energy-dissipating K(+) cycling. The uniporter and the K(+)/H(+) antiporter are in different ways activated by membrane stretching and/or unfolding, resulting in swelling followed by contraction (Schonfeld *et al*.

2003). The basic contention of Shrivastav *et al* (1988) and that of present work is however the same. The exposure of perfused rat liver to depolarizing concentrations of $K^+$(60 mM) by partial substitution of the Nacl in the medium with KCl induces glycogenolysis, respiratory changes and vasoconstriction. These responses were fond to be inhibited 70-80% by 20 μM indomethacin and by 20 micro M bromophenacyl bromides. This suggests that eicosanoid, namely prostaglandins are involved in mediating these effects, and hence the action of potassium ions involves primarily an effect on eicosanoid-producing cells (Kupffer and endothelial cells) within the liver. A five minute pre-exposure of perfused livers to depolarizing concentrations of potassium ions(in the presence of indomethacin) was found to inhibit(by approximately 80%) the influx of Calcium ions induced by the co-administration of 10 nM of glucagons and 10 nM vasopressin. A similar result was observed in isolated hepatocytes.The inhibition was probably not due to a decrease in the concentration of sodium ion in the medium since the substitution of 80mM Nacl with 80mM Cholinechloride resulted in significantly less inhibition (30-40%).These results suggest that under these conditions the influx of Calcium ions in liver occurs through a pathway that is inhibited by high $K^+$ ion concentration and/or a depolarization of plasma membrane (Altin *et al,* 1988).

Effect of $Fe^{2+}$ and ascorbate induced lipid peroxidation on mitochondrial respiration in three and four states (according to Chance) are studied. Peroxidation was shown to result in an increase of oxygen uptake rate in fourth state, and in the KCl containing medium (but not in sucrose medium) it caused a decrease of the oxygen uptake rate in the phosphporylation state, which partially reversed when cytochrome c was added. These effects were observed only after the development of peroxidation (after slow 'flash' of chemiluminescences) and the value of an effect correlated with the content of peroxidation products unsaturated fatty acids. No considerable differences in the damaging effect of peroxidation were observed under incubation of mitochondria in KCl, Nacl and choline containing media (Cheremisina and Vladimirov (1975).

The present study shows that the various main pathways from acetyl Co-A (Krebs cycle, cholesterol synthesis and lipid synthesis) are influenced by minute changes in NaCl concentration and Amino acids. There are two types of amino acids :

(1) which stimulate the major three, pathways from acetyl Co-A and

(2) which inhibit the above three pathways from acetyl Co-A. Certain Amino acids may have applied value in controlling one or more of these pathways from acetyl Co-A in pathological conditions like obesity and convulsive fits.

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