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METABOLIC ASPECTS OF ACUTE TISSUE HYPOXIA DURING EXTRACORPOREAL CIRCULATION AND THEIR MODIFICATION INDUCED BY L-CARNITINE TREATMENT

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Summary: *In this study the authors examine the effects of acute hypoxia due to extracorporeal circulation (ECC) and the role played by L-carnitine treatment on some plasmatic metabolites linked to glycolytic cellular metabolism. To obtain biochemical data, 120 patients in extracorporeal circulation during aortopulmonary bypass surgery were evaluated. The patients received either sodium bicarbonate (40 patients), or L-carnitine during ECC (40 patients) or before and during ECC (40 patients), and plasma samples were collected before ECC, during ECC and after ECC. The levels of lactate and pyruvate showed significant alterations in sodium bicarbonate-treated patients, and there was also a considerable imbalance in the succinate/fumarate ratio. This means that tissue hypoxia due to ECC leads to cellular oxidative damage and to a considerable decrease in the intracellular energy pools. The use of L-carnitine antagonizes the oxidative stress, as is well documented by the levels of plasmatic metabolites which remain confined to normal amounts.*

Introduction

Cellular oxidative and peroxidative damage is essentially the pathogenetic common denominator for hypoxic, ischaemic and reflow pathologies. The various morpho-functional, biochemical and metabolic aspects of cellular response to the altered bioavailability of oxygen and substrates now tend to be interpreted and explained clinically, and it is usual to try to adjust therapeutic plans to the biochemistry that characterizes and induces the development of cellular damage.

In this context, if on the one hand the use of the most advanced technology allows the advantages of studying these phenomena on human models rather than experimental animals, on the other hand one sees the formulating of specific therapeutic principles aimed at counteracting the development of oxidative damage towards irreversible biochemical-metabolic phases or situations. From this arises the opportunity to study the actual preventative and curative incidence of pharmacologically active molecules, with little or no side-effects, on human models in which acute and chronic reflow, hypoxia and ischaemia are the real and putative pathogenetic basis.

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For this purpose, some aspects of the pharmacology inherent in the treatment of biochemical-metabolic tissue and plasmatic damage from hypoxia, ischaemia or reflow can be studied adequately in patients placed under extracorporeal circulation (ECC) for heart surgery.

ECC does, in fact, bring about alterations – although partially reversible and to a modest degree quantitatively – especially against tissue perfusion and the transport of oxygen (1, 2), as amply demonstrated by the increase in peripheral vascular resistance and the releasing of acid metabolites.

Because of this, we believed that ECC could represent an opportune experimental model for studying the response to a situation of relative tissue hypoxia and the subsequent reflow, through the analysis of the behaviour of certain plasmatic metabolites directly correlated to cellular enzymatic activity.

Furthermore, we wished to verify the possibility of opposing the consequence of this hypoperfusive situation by the use of L-carnitine (3-hydroxy-4N-trimethyl-aminobutyrate), which has already shown a protective capacity during acute hypoxia, especially during the stage of residual reversibility of oxidative damage, which seems to coincide with a period of enzymatic adaptation to the deficit of oxygen and substrates (3–7).

With this study we have therefore wished to focus our attention on the plasmatic levels of lactate and pyruvate, taken as an index of tissular glycidic metabolism, and of succinate and fumarate, understood as a function of parametrization of the redox capacity of the Krebs cycle with respect to the activity of the mitochondrial respiratory chain and thus indirectly of the endocellular energy capacity.

Materials and methods

The study, conducted double-blind and randomized, took into consideration 120 patients (102 males and 18 females), between 50 and 55 years of age, weighing 70 ± 6 kg, and undergoing aortocoronary bypass operations with ECC (Table I). The subjects were divided at random into 3 groups of 40 patients each. Sodium bicarbonate was given to group A (control) at the same times and in the same volumes as L-carnitine, which was given to the patients in groups B and C. Group B was treated with L-carnitine in a dose of 4 g in bolus i.v. given right after the induction of anaesthesia, followed by additional i.v. infusion of 2 g, carried out every 6 h for a period of twelve hours. Group C received L-carnitine at the same times and in the same doses as group B, in addition to a pretreatment of 3 g in infusion 12 h before surgery. The placebo was given using the same procedure as for group C: in particular, a 0.9% chlorinated solution of 30 ml was given 12 h before surgery, followed by 40 ml at the induction of anaesthesia and 20 ml every 6 h thereafter.

The patients were selected according to strict inclusion criteria, which excluded subjects affected by episodes of infarction in progress, those with neoplasms and those undergoing treatment with anti-oxidants and psychopharmaceuticals. At the time of inclusion in the protocol, the anamnestic and anthropometric data of each patient were examined and recorded, in order to obtain a group of 120 subjects as homogeneous as possible with respect to physio-pathological characteristics.

For anaesthesia, the probands were then premedicated by administering Diazepam (0.1 mg/kg p.o.) and morphine (0.12 mg/kg) i.m. The induction of anaesthesia called for the use (i.v.) of Fentanyl (10–20 µg/kg) and Diazepam (0.20–0.25 mg/kg).

Table I *Notes on patients and ECC.*

	Group A	Group B	Group C
Patients	40	40	40
Age	62 ± 5.9	61 ± 4.9	63 ± 3.6
BSA (m^2)	1.77 ± 0.06	1.74 ± 0.09	1.77 ± 0.07
ECC (min)	118 ± 42	114 ± 31	144 ± 41
Clamping (min)	76 ± 35	77 ± 35	73 ± 30

Mioresolution was obtained by Pancuronium bromide (0.1 mg/kg) administered i.v.

The maintaining of the desired levels of anaesthesia was carried out by the i.v. administration of repeated doses of Fentanyl up to a maximum dosage of approximately 80–100 µg/kg, along with the supplying of a gaseous mixture of oxygen and air (FI02 = 0.60). The muscular relaxation was maintained by hemidoses of Pancuronium bromide, administered i.v. as needed.

The ECC (115 ± 18 min) was carried out (after the systemic heparinization of the patient (3 mg/kg) by cannulation of the ascending aorta and the right atrium with a single atrio-caval cannula, using SARNS occlusive pumps and Terumo hollow-fibre oxygenators. Priming of the ECC circuit was done with 2–3 ml/kg of balanced polyelectrolytic crystalloidal solution (pH-7.4) with the addition of 50% glucosate or 5% PPS.

The cardiopulmonary bypass made it possible to guarantee a cardiac index of approximately 2.0 ± 0.5 l/min/m, with a perfusion pressure of around 60 mmHg, when necessary resorting to the use of vasodilators (sodium nitroprusside, nitroglycerin).

The minimum temperature reached in the patients was 25.8 ± 3°C. Myocardial protection during aortic clamping (75 ± 8 minutes) was guaranteed by the administering of St. Thomas hyperpotassic cardioplegic solution at 4°C every 20–30 min, supplemented by surface hypothermia of the heart obtained by irrigation of the pericardiac sac with cold physiological solution.

During the aortocoronary bypass the lungs were not ventilated; however a gaseous mixture of oxygen and air was administered so as to maintain a static insufflation pressure of 5 cmH_2O. All of the patients were extubated about 12 h after surgery, having reached stable haemodynamic and ventilation conditions.

The plasmatic levels of lactate, pyruvate, fumarate and succinate were measured from venous blood samples, and the lactate/pyruvate and succinate/fumarate ratios were calculated at the following times: 24 h previous to the induction of anaesthesia (T1), 60 min after the start of ECC (T2), 10 min after termination of ECC (T3) and 12 h after the surgery was completed (T4).

These determinations, obtained in triplicate, were carried out using the techniques of J.R. Williamson (8) for plasmatic levels of succinate and fumarate, and of F. Noll (9) and R. Czok (10) for lactate and pyruvate. The data obtained (presented as means and S.D.) were then evaluated using Student's t test in order to determine statistical significance.

Table II *Plasmatic levels of lactate, pyruvate, succinate and fumarate in ECC patients, treated with $NaHCO_3$ (control Group A), treated with L-carnitine (Group B), and pretreated and treated with carnitine (Group C).*

	Group A	Group B	Group C
Lactate (mg/dl)			
T1	9.8 ± 4.0		10.6 ± 4.6
T2	28.1 ± 14.0		20.5 ± 12.7 (**)
T3	62.2 ± 26.1		30.5 ± 17.7 (*)
T4	56.1 ± 24.6		20.9 ± 14.0 (*)
Pyruvate (mg/dl)			
T1	1.4 ± 0.5	1.1 ± 0.6	1.3 ± 0.9
T2	0.7 ± 0.5	1.2 ± 0.6 (*)	1.5 ± 1.0 (*)
T3	0.4 ± 0.3	1.4 ± 0.7 (*)	1.8 ± 0.9 (*)
T4	0.9 ± 0.4	1.7 ± 0.7 (*)	2.1 ± 0.9 (*)
Succinate (mg/dl)			
T1	0.9 ± 0.4	1.0 ± 0.4	0.9 ± 0.4
T2	1.7 ± 1.2	1.1 ± 0.4 (*)	0.9 ± 0.5 (*)
T3	3.1 ± 1.9	1.0 ± 0.4 (*)	0.8 ± 0.4 (*)
T4	2.5 ± 1.6	0.9 ± 0.3 (*)	0.9 ± 0.3 (*)
Fumarate (mg/dl)			
T1	0.8 ± 0.4	0.9 ± 0.4	0.8 ± 0.4
T2	0.6 ± 0.4	0.8 ± 0.4 (*)	0.6 ± 0.3 (*)
T3	0.2 ± 0.2	0.8 ± 0.5 (*)	0.7 ± 0.4 (*)
T4	0.6 ± 0.4	1.0 ± 0.3 (*)	1.0 – 0.4 (*)

* $p < 0.0001$; ** $p < 0.0015$.
T1, 24 h before anaesthesia; T2, 60 min after ECC started; T3, 10 min after ECC ended; T4, 12 h after completion of surgery.

Results

The results obtained from this study (Table II) have made it possible to ascertain that the reduction, compared to the norm, of the cardiac index and thus of the flows resulting from ECC actually involves the start of oxidative damage – although oscillating in most cases – within the limits of reversibility and therefore not causing imbalances great enough to jeopardize the clinical situation.

The biochemical-metabolic alteration which takes place during ECC is shown by the behaviour of the plasmatic concentrations of lactate (Fig. 1) and of pyruvate (Fig. 2) in the control subjects (group A), the levels of which undergo significant variations in an opposite manner. In particular, there is a progressive increase in the concentrations of lactate beginning with T1 (9.8 ± 4.0 mg dl) up to T3 (62.2 ± 26.1 mg/dl) with high levels persisting even 12 h after surgery (T4 = 56.1 ± 24.6 mg/dl).

Pyruvate, instead, behaved in an opposite manner, showing a progressive reduction of its plasmatic concentration from T1 (1.4 ± 0.5 mg/dl) up to T3 (0.4 ± 0.3 mg/dl), then returning to values close to those found in the preoperative period (T4 = 0.9 ± 0.4 mg/dl).

The patients treated with L-carnitine behaved differently: in both groups the increase in lactate concentrations was significantly lower (p 0.0015 and p 0.001) as compared to group A (group B: T2 = 18.3 ± 10.3 mg/dl; T3 = 26.9 ± 14.2 mg/dl; group C:

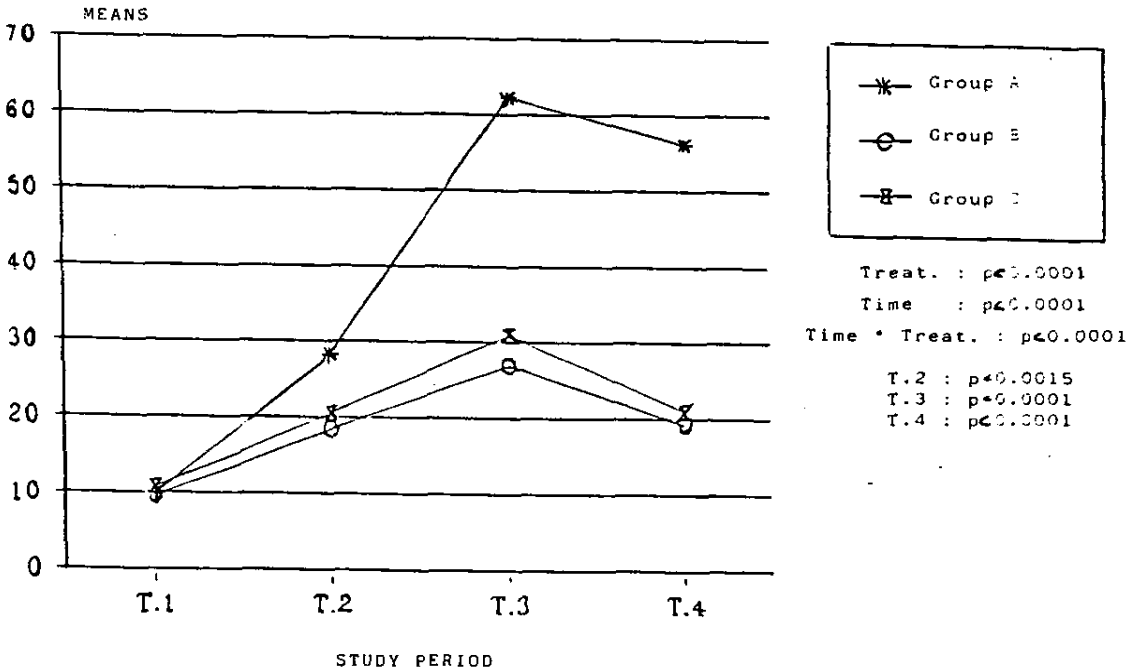

Fig. 1 Plasmatic lactate concentrations (mg/100 ml) in the control group (Group A) and in the subjects treated with L-carnitine (Group B and Group C)

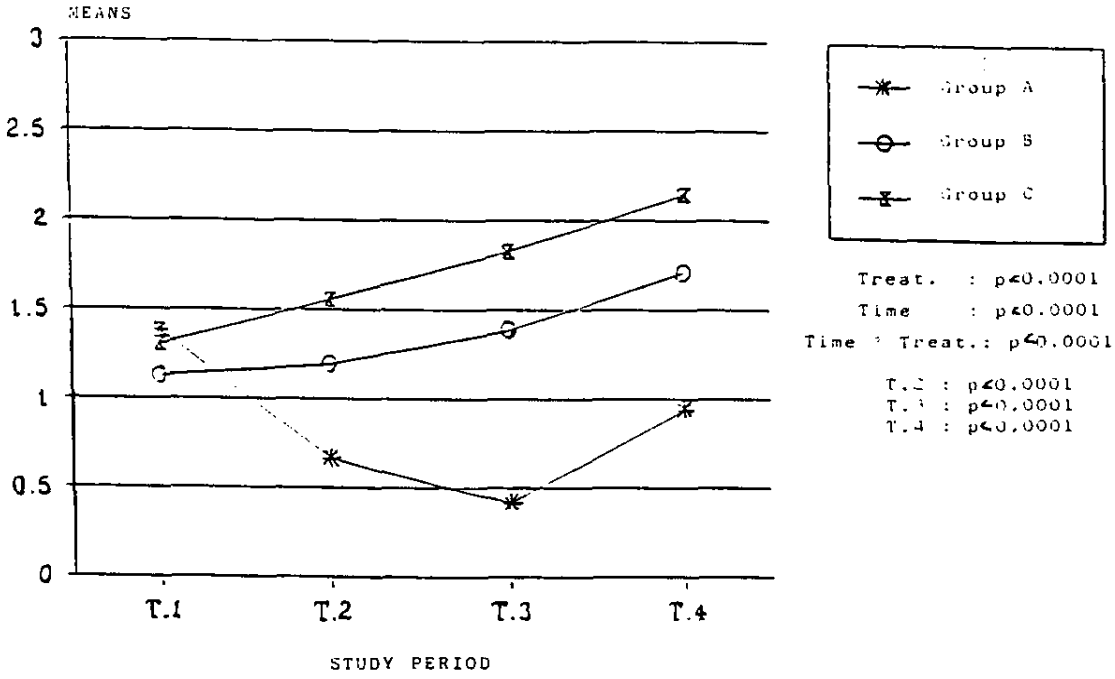

Fig. 2 Plasmatic pyruvate concentrations (mg/100 ml) in the control group (Group A) and in the subjects treated with L-carnitine (Group B and Group C).

T2 = 20.5 ± 11.7 mg/dl; T3 = 30.5 ± 17.7 mg/dl).

Different behaviour was also shown by pyruvate in the patients treated with L-carnitine. Contrary to that which happened with the control group, he pyruvate underwent a progressive, constant and significant increase: from 1.1 ± 0.6 mg/dl (T1) to 1.7 ± 0.7 mg/dl (T4) in group B, and from 1.3 ± 0.9 mg/dl (T1) to 2.1 ± 0.9 mg/dl (T4) in group C.

Regarding the behaviour of plasmatic concentrations of succinate, in the control group there was a progressive increase similar to that of the lactate, from preoperative values (T1 = 0.9 ± 0.4 mg/dl) up until the end of ECC (T3 = 3.1 ± 1.9 mg/dl), remaining high even 12 h after surgery was completed (T4 = 2.5 ± 1.6 mg/dl). This metabolite shows, on the other hand, considerably more stable behavior in the two groups treated with L-carnitine (Fig. 4).

In the control group, the behaviour of fumarate mirrored that of pyruvate, with a progressive reduction of its plasmatic concentrations, from basal values (T1 = 0.8 ± 0.4 mg/dl) up to the period immediately following ECC (T3 = 0.2 ± 0.2 mg/dl), then rising again 12 h after surgery to values near the preoperative values (T4 = 0.6 ± 0.4 mg/dl). The groups treated with L-carnitine did not show any appreciable variations in the plasmatic concentrations of this metabolite (Fig. 5).

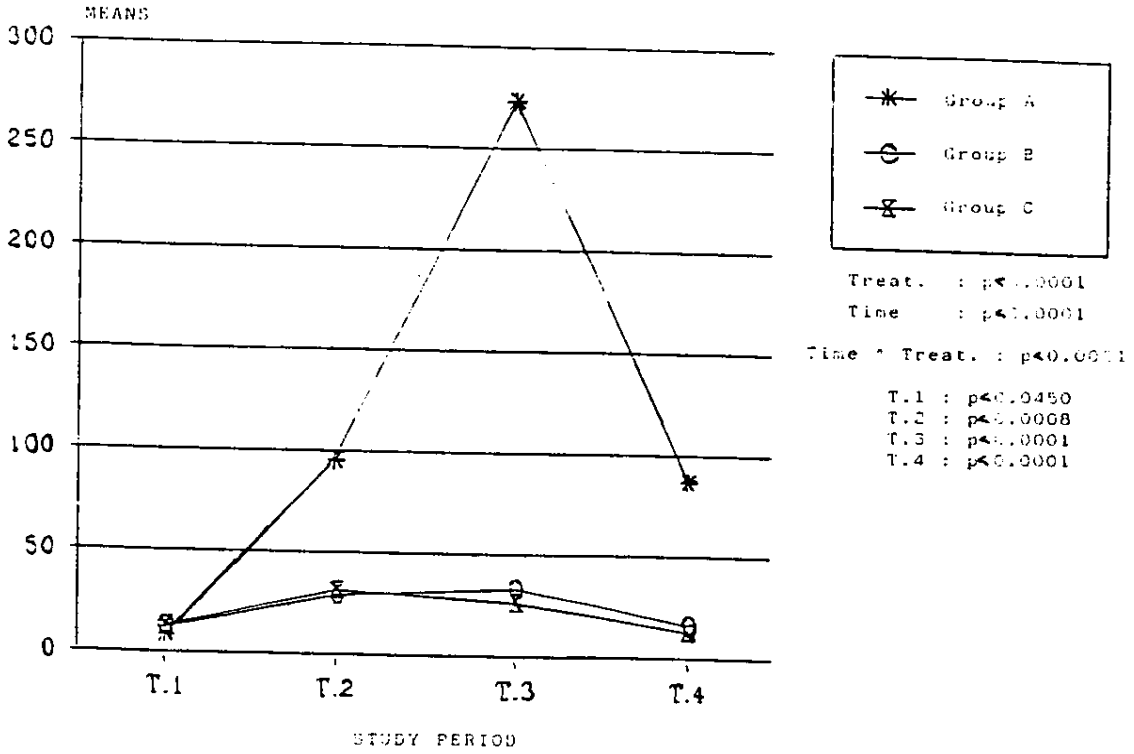

Fig. 3 Lactate-pyruvate ratio in the control group (Group A) and in the subjects treated with L-carnitine (Group B and Group C).

Discussion

The fact that ECC causes a condition of tissue hypoperfusion and that the cellular hypoxia deriving from it turns into a situation of oxidative stress does not seem surprising. What our study, however, indicates is the fact that the oxidative damage suffered by the cells during cardiopulmonary bypass surgery appears to be less negligible than it had seemed to be.

In fact, in the control subjects, the levels of lactate and pyruvate, as well as the considerable alteration of their ratio (Fig. 5), reveal a frankly anaerobic metabolic condition, indicating that the partial reduction of blood flows following ECC affects both the glycidic metabolism and the mitochondrial functional capacity. This supports the hypothesis that the endocellular enzymatic systems, in their totality, are those that suffer the greatest damage from the deficit of oxygen and substrates.

Furthermore, the regression of this alteration of the biochemistry appears to be much less rapid than its onset, as is demonstrated by the fact that the plasmatic values of ths metabolites remain changed even 12 h after surgery. From a clinical point of view, this means that this state of cellular

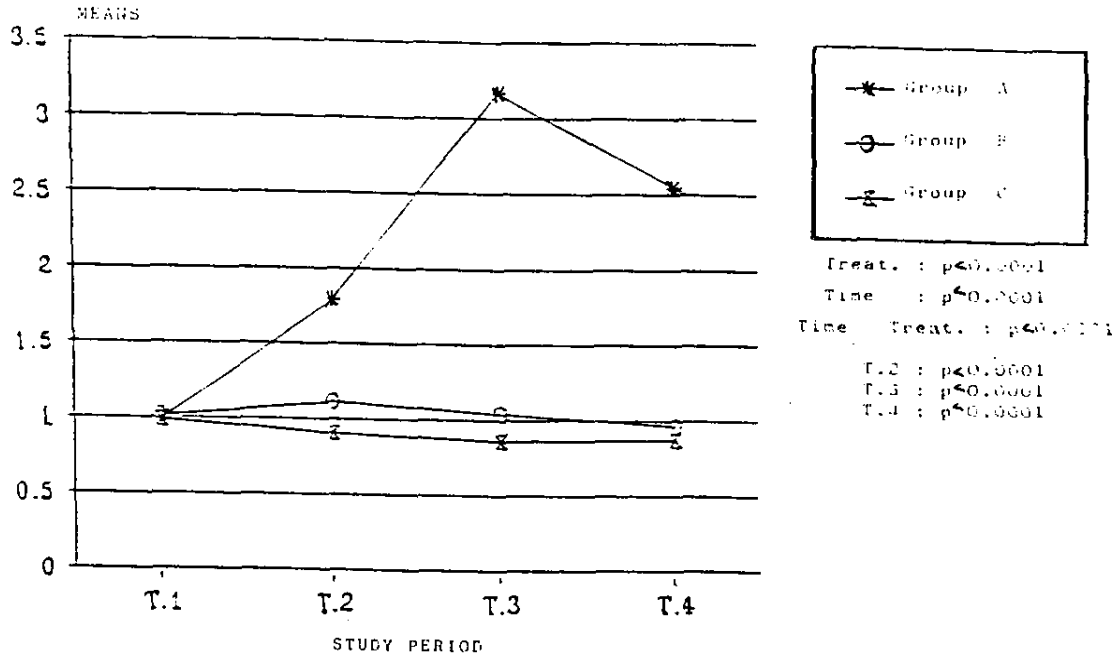

Fig. 4 Plasmatic succinate concentrations. (mg/100ml) in the control group (Group A) and in the subjects treated with L-carnitine (Group B and Group C).

stress could constitute the pathogenetic basis for any complications arising even some time after open-heart surgery.

More harmful still, in the sense of an oxidative damage from hypoxia, is the partial inactivation of the electroionic synergisms between the tricarboxylic acid cycle and the enzymatic complexes of the mitochondrial respiratory chain, as attested in the patients of group A by the altered succinate/fumarate ratio (Fig. 6).

Moreover, the decreased enzymatic capacity of the "succinate-dehydrogenase" complex brings about a consequential inactivation of the other enzymatic complexes of the mitochondrial respiratory chain. In similar circumstances, the marked reduction of energy substrates which, having been consumed by cell metabolism, are regenerated at a level inadequate for enzymatic requirements, could be a sign of the endocellular production of elements of oxy-radical structure and function. Triggering well-known peroxidative mechanisms, these elements tend to alter, at times irreversibly, the morpho-functional balances of the cells.

The metabolic responses in the subjects of the two groups treated with L-carnitine seem notably different: concerning this, it should be emphasized that no substantial differences appeared between the two treatment methods carried out by us. In these patients, in fact, there was a constant tendency towards the normalization of the plasmatic levels of the metabolites being studied and of their quantitative ratios (Figs. 3 & 6). This was to such a

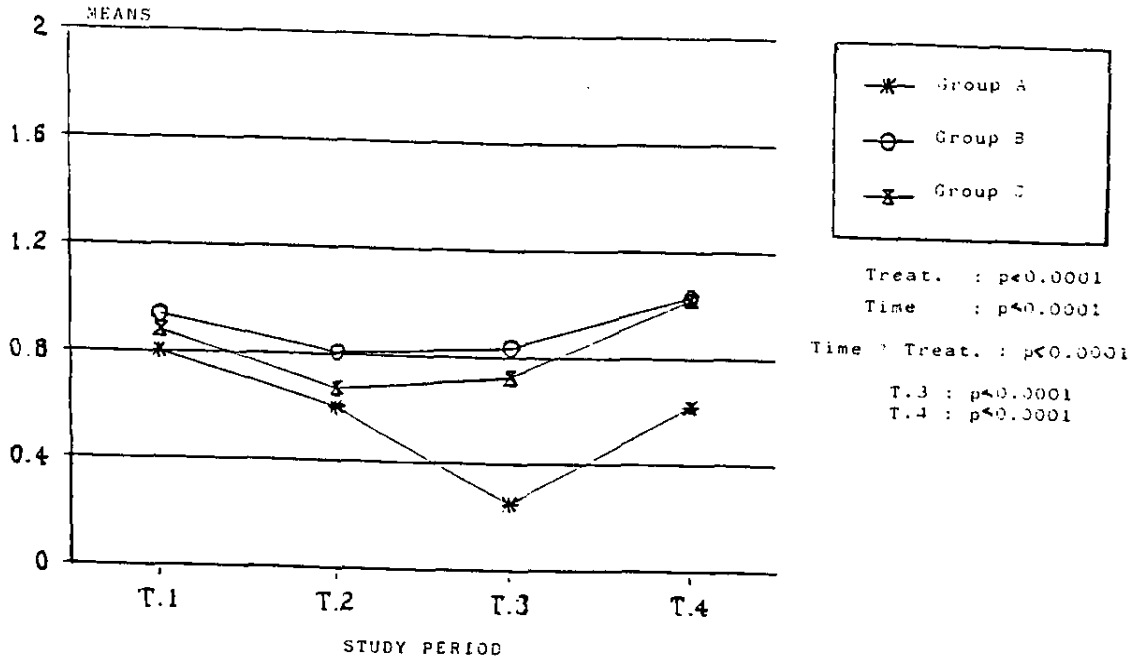

Fig. 5 Plasmatic fumarate concentrations (mg/100ml) in the control group (Group A) and in the subjects treated with L-carnitine (Group B and Group C).

degree that in similar circumstances the stress from hypoxia remained within limits that, if not exactly physiological, at least were not drastically pathological. Therefore, since this protective action of L-carnitine cannot be focussed on the kinetics of specific enzymatic complexes, it is presumable that the determining factor in the molecule's distinctive role is its ability to keep endocellular concentrations of energy substrates sufficient for sustaining the aforesaid kinetics and related metabolisms.

This fact, previously hypothesized by other authors (7) in experimental situations, seems to be of great importance and interest in human hypoxic pathology, endowing L-carnitine with a corrective value definitely much more specific and effective than other therapeutic defenses being used. In fact, this endogenous molecule, which is practically devoid of side-effects, seems to act protectively towards the hypoxic cell, having the effect on the endocellular pathogenetic base of oxidative damage of keeping it within the limits of reversibility. In other words, L-carnitine seems to act neither as a detoxicant, nor as a oxy-radical "scavenger", nor as an inactivator of abnormal biohumoral mediators; rather, it presumably has only a rebalancing role against excessive metabolic cell imbalances, which, due to their magnitude and prolongation, could become chronically rooted.

The results of this study, which confirm the suppositions of our previous investigations (5, 6), therefore seem to prove that L-carnitine, when properly used, finds its most specific and effective application in the acute hypoxic pathology of man.

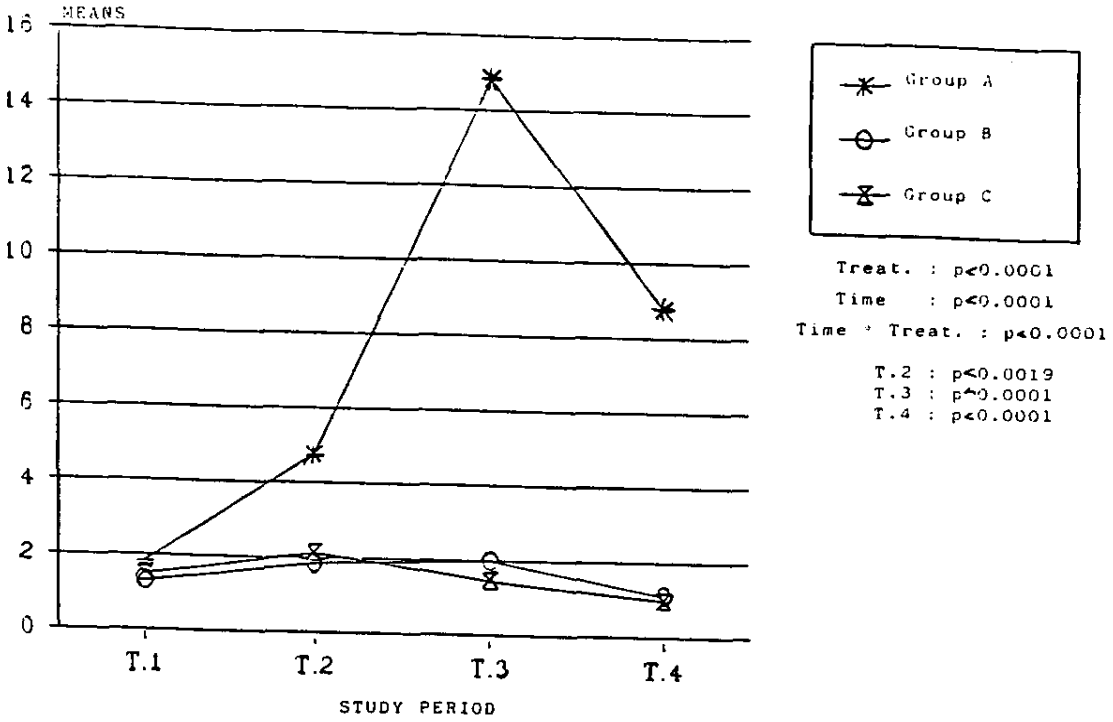

Fig. 6 Succinate/fumarate ratio in the control (Group A) and in the subjects treated with L-carnitine (Group B and Group C).

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