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Effects of Vitamin E Deficiency on GSH-Induced Swelling of Rat Liver Mitochondria[1, 2] (38662)

SYDNEY O. ALOZIE,[3] LEGRANDE C. ELLIS, RALPH M. JOHNSON, AND JOSEPH C. STREET

Department of Chemistry and Biochemistry Utah State University, Logan, Utah 84322

The role of vitamin E (*dl*-α-tocopherol) in cellular metabolism is not clearly understood at the present time, but its participation in the mitochondrial electron transport system either as a factor or as a structural agent has been suggested (1–3). Accelerated respiratory decline observed in liver slices during prolonged incubations is one of the manifestations of liver mitochondria deficient in α-tocopherol (4, 5).

Identification of α-tocopherol with the inner mitochondrial membrane (6) has led to the speculation that it functions in membrane metabolism, especially membrane permeability (7). On this basis the current investigations were initiated to study the effect of vitamin E on the respiration-dependent swelling of rat liver mitochondria caused by reduced glutathione (GSH).

Materials and Methods. Male Sprague–Dawley rats (Horton Laboratories, Oakland, CA) were raised from 21 days of age on a basal casein diet deficient in α-tocopherol (8). Control animals were fed the same diet supplemented with α-tocopherol. Blood samples were taken weekly via the tail vein and assayed for α-tocopherol deficiency using the dialuric acid hemolysis test (9). The animals were sacrificed when the differences in red cell hemolysis between the control and deficient groups were 100%. α-tocopherol was administered (2 mg/rat per day in 0.5 ml corn oil) by intubation to some of the deficient rats for the terminal 4 days while being fed the deficient diet. The control rats were given 0.5 ml corn oil per day for 4 days.

Mitochondria were prepared in 0.25 sucrose, containing 0.02 *M* Tris–HCl buffer, pH 7.4, according to the method of Sotocassa *et al.* (10). Care was taken to avoid contamination of the mitochondrial fraction by the nuclear fraction, microsomal fraction, and fluffy layer. The mitochondria were resuspended in fresh 0.25 *M* sucrose at 0° at concentrations required to give an initial optical density of approximately 0.6–0.7 when 0.1 ml of the suspension was transferred to 2.9 ml of the medium used to measure swelling. Mitochondrial swelling was determined by measuring the decrease in optical density at 520 nm (11) at room temperature with a recording spectrophotometer (Perkin-Elmer Coleman Model 124). Exact test conditions for the specific assays are described in the legends of the figures. Experiments were carried out with freshly prepared liver mitochondria from both normal and α-tocopherol deficient rats, as mitochondria which were allowed to stand for several hours at 0° beyond their preparation did not respond in a manner similar to those from freshly prepared tissues.

Results. Deficient mitochondria resuspended in either 0.3 *M* sucrose or 0.154 KCl at room temperature swelled much faster than those from control animals (Fig. 1). The rate of swelling was faster in sucrose (0.3 *M*) for both the control and deficient mitochondria than in KCl (0.154 *M*). GSH greatly enhanced the swelling tendency of the deficient mitochondria (compare Fig. 2 to Fig. 1).

Several agents were shown to influence GSH mediated swelling of liver mitochondria. These were: ATP, polyvinyl pyrrolidone (PVP, a high molecular weight polymer), and the respiratory inhibitors—cyanide and

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[3] Present address: Interdepartmental Toxicology Program, Nutrition and Food Science Department, UMC 48, Utah State University, Logan, UT 84322.

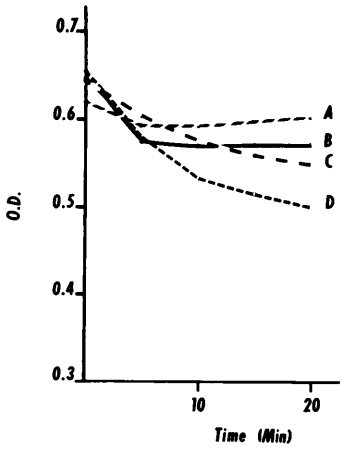

FIG. 1. The effect of incubation media and α-tocopherol deficiency on mitochondrial swelling of rat liver preparations. Ten animals were used per observation. A, Control mitochondria incubated in 0.154 *M* KCl; B, α-tocopherol deficient mitochondria incubated in 0.154 *M* KCl; C, Control mitochondria incubated in 0.30 *M* sucrose; and D, α-tocopherol deficient mitochondria incubated in 0.30 *M* sucrose. Both media were buffered at pH 7.4 with 0.02 *M* Tris-HCl.

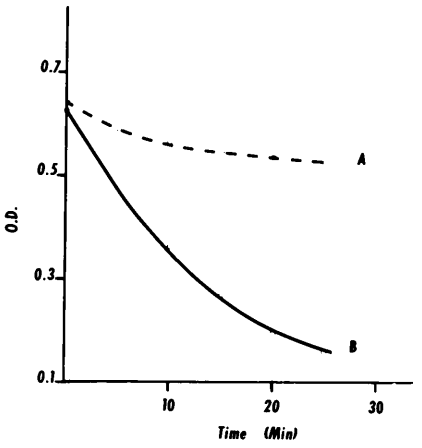

FIG. 2. GSH-induced swelling of liver mitochondria from rats on α-tocopherol deficient and supplemented casein diets. Eight animals were used per observation. The swelling medium consisted of 0.3 *M* sucrose buffered at pH 7.4 with 0.02 *M* Tris-HCl. A, Control mitochondria + 0.05 *M* GSH; B, α-tocopherol deficient mitochondria + 0.05 *M* GSH.

azide. GSH-induced swelling was completely eliminated when mitochondria were left longer than two hours at 0° in the suspending medium. ATP completely reversed the GSH-induced swelling action of both control and deficient mitochondria (Fig. 3).

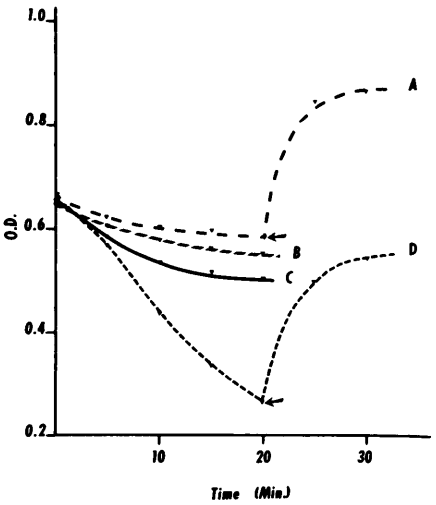

FIG. 3. The reversal of swelling of liver mitochondria by ATP. The swelling medium consisted of 0.3 *M* sucrose buffered at pH 7.4 with 0.02 *M* Tris-HCl. Eight animals were used per observation. A, Control mitochondria + 0.05 *M* GSH; B, Control mitochondria in 0.3 *M* sucrose (spontaneous swelling); C, Deficient mitochondria in 0.3 *M* sucrose (spontaneous swelling); D, α-tocopherol deficient + 0.05 *M* GSH. The arrows indicate the time of addition of ATP to the media (final concentration 0.01 *M*).

The data (Fig. 4) show that a 7.7% solution of PVP was effective in reversing swelling induced by GSH in both control and deficient mitochondria. The extent to which the swelling of mitochondria was reversed diminished with time.

In view of the marked swelling tendency of liver mitochondria obtained from α-tocopherol deficient rats, the effects of several respiratory inhibitors were investigated. By maintaining all the electron carriers in the reduced state with potassium cyanide (0.001 *M*), complete inhibition of the GSH-induced swelling of liver mitochondria from both the control and deficient animals was observed. Swelling was not inhibited by sodium azide (0.001 *M*), but a much reduced swelling of the mitochondria was observed (Fig. 5).

The difference in swelling between the deficient and the control mitochondria disappeared following a short course of orally administering α-tocopherol to the rats on the deficient diet (Fig. 6).

Discussion. Our data demonstrate that the α-tocopherol deficient state influences the

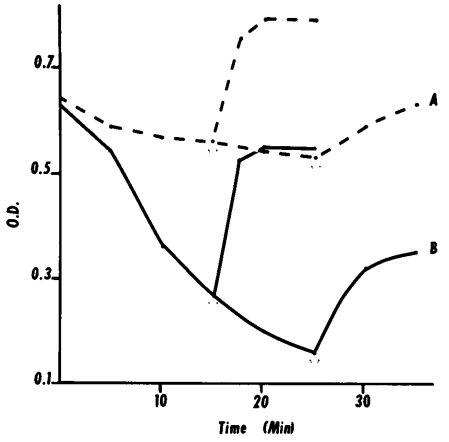

FIG. 4. The effect of high particle weight solute (PVP) on GSH-induced swelling of rat liver mitochondria. The swelling medium consisted of 0.3 *M* sucrose buffered at pH 7.4 with 0.02 *M* Tris–HCl. PVP, 7.7% (w/v), was added to the media at either 15 or 26 min as indicated by the arrows. Eight animals were used per observation. A, Control mitochondria + 0.05 *M* GSH; and B, α-tocopherol deficient mitochondria + 0.05 *M* GSH.

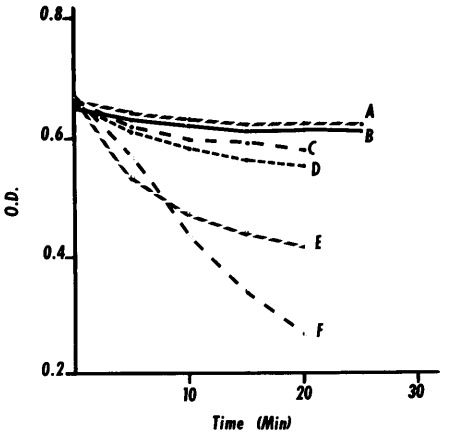

FIG. 5. The effect of the respiratory inhibitors, cyanide and azide, on GSH-induced swelling of rat liver mitochondria. The swelling medium consisted of 0.3 *M* sucrose and 0.05 *M* GSH buffered at pH 7.4 with 0.02 *M* Tris–HCl. Eight animals were used per observation. A, Control mitochondria + 0.001 *M* cyanide; B, α-tocopherol deficient mitochondria + 0.001 *M* sodium cyanide; C, Control mitochondria; D, Control mitochondria + 0.001 *M* sodium azide; E, α-tocopherol deficient mitochondria + 0.001 *M* sodium azide; and F, α-tocopherol deficient mitochondria.

mitochondrial swelling of rat livers under various experimental conditions.

Swelling in normal mitochondria has been assumed to be dependent on the activities of the electron transport system (12, 13). These data showed that inhibitors of this system (i.e., cyanide and azide), prevented or reduced GSH-induced swelling of normal and tocopherol-deficient liver mitochondria. These results suggest that deficient mitochondria, similar to the situation seen in normal mitochondria, do not swell in the absence of electron transport activity and that the phenomenon is dependent on a functional respiratory chain. Other workers have suggested that mitochondrial swelling might be mediated at the cytochrome C level (14, 15). Our observations with cyanide corroborate this conclusion. Reversal by ATP or GSH-induced swelling of liver mitochondria from rats on α-tocopherol deficient diets indicates an involvement of this vitamin in the respiratory chain and possibly the coupling mechanism within the mitochondria.

During the thiol-induced mitochondrial swelling, S-S groups essential for membrane integrity are known to be reduced to SH groups (15). In the following contraction

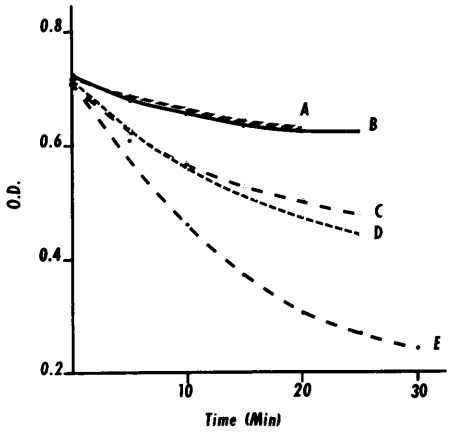

FIG. 6. The effect of orally administered α-tocopherol on rat liver mitochondrial swelling. The swelling medium consisted of 0.3 *M* sucrose buffered at pH 7.4 with 0.02 *M* Tris–HCl. Five animals were used per observation. Two mg of α-tocopherol were administered in 0.5 ml corn oil for four days prior to sacrifice. A, Control mitochondria (spontaneous swelling); B, α-tocopherol deficient mitochondria + α-tocopherol; C, Control mitochondria + 0.05 *M* GSH; D, α-tocopherol deficient + oral α-tocopherol + 0.05 *M* GSH; and E, α-tocopherol deficient mitochondria + 0.05 *M* GSH.

process, a reconstitution of the membrane S-S groups by an unidentified endogenous oxidant, with attendent enzyme catalysis, is assumed to take place (15). The possibility of the involvement of α-tocopherol in the above mechanism cannot be ruled out, on the basis of the swelling tendencies of mitochondria from either normal or α-tocopherol deficient livers.

Inhibition of mitochondrial swelling by PVP has been attributed to the inability of this solute to pass through the mitochondrial membrane(s), thereby producing an osmotic pressure difference between the extra- and intra-mitochondrial phases (11). The reversal of the GSH-induced swelling of mitochondria by PVP is suggestive of a conversion of mitochondrial membranes to "passive osmometers" by α-tocopherol.

Swiercyzinski *et al.* (3) have suggested that an α-tocopherol effect on mitochondrial membrane might be due to a chemical interaction with the molecular arrangement of mitochondrial membrane. This interaction, therefore, might regulate permeability. Such interaction could also alter the relationship between respiratory chain enzymes and membrane properties (16). The resultant effect of the above could be an alteration in the respiration and phosphorylation rates, as well as changes in the permeability or contractility of membranes during α-tocopherol deficiency (16).

Of significance, in this study, is the relatively rapid reversal of mitochondrial swelling observed after the oral administration of α-tocopherol for four days indicative of a possible direct effect of α-tocopherol on this phenomenon. The present studies indicate that in the α-tocopherol deficient liver mitochondria, (a) swelling is readily induced by GSH, and (b) the resultant swelling is sensitive to respiratory inhibitors. These findings may suggest a role for α-tocopherol in the respiration of rat liver mitochondria. Nevertheless, the actual role played by this vitamin in the function of mitochondria needs further clarification.

Summary. Liver mitochondria from α-tocopherol deficient rats swell more rapidly, both spontaneously and in the presence of GSH, than those from control animals. The increased swelling of deficient mitochondria induced by GSH was completely eliminated when mitochondria were left longer than two hours at 0° in the suspending medium. GSH-induced swelling of liver mitochondria from α-tocopherol deficient rats is reversed by ATP, polyvinyl pyrrolidone (PVP), and by oral administration of α-tocopherol (2 mg/day per rat). Swelling of mitochondria in the above system was completely inhibited by the respiratory chain inhibitor, cyanide (0.001 *M*), and partially by azide (0.001 *M*). We suggest that the swelling tendency of liver mitochondria from α-tocopherol deficient rats might be associated with an altered respiratory mechanism and/or an alteration in membrane permeability.

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