ins. Homolytic cleavage of the CCl_3–Cl bond evidently produces such a shower of CCl_3 radicals that any protection effort by peroxidase is overwhelmed. Rao et al.[16] also reported that lower activities of lipid peroxidase and nucleotide oxidase might be favorable for optimum aflatoxin biosynthesis, but they started from presuppositions quite different from ours. According to them, in fact, lipid peroxidation was increased within the mycelium under conditions of reduced aflatoxin production; under these conditions lipid synthesis and growth are enhanced and greater availability of lipids would elevate lipid peroxidase activity.

* Acknowledgments. Supported by 'Cassa di Risparmio di Roma', funds from Ministry of Education and Faculty of Science of 'La Sapienza' Rome University.

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0014-4754/84/020191-03$1.50 + 0.20/0

Physiological state of submitochondrial particles and their susceptibility to Triton X-100[1]

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Summary. The solubilizing effect of Triton X-100 on beef heart submitochondrial particles (ETP_H) has been studied under various physiological conditions. Coupled, uncoupled and azide-inhibited ETP_H particles have been studied. Quantitative and qualitative differences are found in the proteins solubilized by the detergent from ETP_H particles under the various conditions tested.

There have been various reports of differences in susceptibility of membranous systems to the solubilizing action of detergents, according to the physiological state of the membranes[1-4]. Some of these studies have been carried out on microorganisms, or microbial membranes, generally by inducing inhibited states through cyanide or sodium azide. Detergent action was usually assessed by the release of cytoplasmic contents.

Studies on the effect of detergents on mitochondria and mitochondrial membranes have also been carried out, in this and in other laboratories[5-7]. It was found that Triton X-100 was very useful in this respect, because of its selective solubilizing action and mild effect on enzyme activities at low concentrations, together with a high solubilizing power at higher detergent/membrane ratios.

The well-known conformational changes accompanying variations in the mitochondrial physiological state[8], together with the selectivity of Triton X-100 action[6,9] and indications that the accessibility of membrane lipids and proteins to externally added reagents varies with energization/deenergization processes[4,8,10-12] suggest that mitochondria in different physiological states may behave differently towards detergents. However, this hypothesis was difficult to test with the usual lengthy methods (centrifugation, dialysis, etc.) of separating the solubilized from the non-solubilized membrane fraction, because of the problems of maintaining mitochondria in a defined functional state for such long time intervals. In this paper, we describe a fast and efficient filtration method to achieve that aim, and we also record the quantitative and qualitative differences found in Triton X-100 solubilized protein from mitochondria in different physiological states.

Materials and methods. Beef heart submitochondrial particles (ETP_H) were prepared according to[13]. They were washed and resuspended in a few ml of 0.25 M sucrose, 0.01 M Mg^{2+} and 10 mM Tris-HCl, pH 7.2.

The physiological state of ETP_H was tested by means of a Clark-type oxygen electrode (Rank Bros., Bottisham, U.K.) using an assay medium containing 2 mM K_2HPO_4, 75 mM KCl, 0.5 mM EGTA, 1 μM rotenone and 10 mM Tris-HCl (pH 7.0). With succinate-maintained respiration, respiratory control ratios were 1.2-1.4 at 38 °C. The ETP_H suspension was diluted to a final concentration of 1 mg protein/ml in the assay medium indicated above. 'Respiring' submitochondrial particles (1 mg protein/ml) were assayed in the presence of 20 mM succinate and 0.6 mM ADP; 8 μM carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was added in order to obtain 'uncoupled' ETP_H; finally, 'inhibited' submitochondrial preparations contained 20 mM succinate, 0.6 mM ADP and 20 mM sodium azide. Polarographic observations showed that the submitochondrial preparations remained in their respective functional states for longer than 1 min.

The different ETP_H suspensions were treated with the required amounts of Triton X-100 in order to obtain final detergent concentrations ranging from 10^{-5} to 10^{-3} M, and

incubated at 25 °C for 1 min. They were then immediately filtered through two filters in series, (0.08 μm (Whatman GF/A) and 0.22 μm (Millipore GSWPO 1300) pore diameter), mounted on a Millipore Swinnex-13 support. No more than 10 sec elapsed between the end of the incubation and the end of the filtration step.

Proteins were determined by the method of Lowry et al.[14] with the modification of Wang and Smith[15]. Polypeptides were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE) according to Fairbanks et al.[16]. The gel cylinders (75 mm long) were stained with Coomassie brilliant blue and scanned at 575 nm in a UV-5260 Beckman spectrophotometer equipped with a densitometry accessory.

Results. The amount of mitochondrial protein solubilized by a given concentration of Triton X-100 depends in many cases on the functional state of the submitochondrial particles, as can be seen in figure 1. Uncoupled and inhibited submitochondrial particles behave in the same way, and both differently from the actively respiring coupled ETP_H. The latter are significantly less susceptible to the solubilizing action of 10^{-4} M Triton X-100. The opposite happens with 6×10^{-4} M detergent when more protein is solubilized from coupled ETP_H than from any of the other 2 preparations.

The influence of the physiological state of submitochondrial particles on protein solubilization is not only quantitative, but also qualitative; different polypeptide profiles are seen when the solubilized fractions of coupled, uncoupled and inhibited mitochondria are analyzed by SDS-PAGE (fig. 2). Examining the fractions obtained with 6×10^{-4} M Triton X-100 it can be seen that, in accordance with the quantitative data (fig. 1), the polypeptide compositions of uncoupled and inhibited ETP_H are quite similar, and both different from that of coupled submitochondrial particles.

Discussion. The present work demonstrates the influence of the physiological state of mitochondria on their susceptibility towards Triton X-100 solubilization. There have been previous reports of similar observations on other membranous systems[1-4] but this is the first to deal with a cell fraction well-defined from the chemical, physiological and ultrastructural points of view, such as beef heart ETP_H submitochondrial particles[13]. Our observations were made possible by the development of a fast filtration technique for the separation of the solubilized from the non-solubilized mitochondrial fractions after detergent action. This led to the additional advantage over previous studies that we were able to isolate the proteins solubilized by the detergent, and consequently to analyze qualitatively this mitochondrial subfraction. It should be noted in this respect that this solubilized fraction is defined only according to filtration criteria, and in fact it does contain some particulate material[7].

We have limited our study to submitochondrial particles treated for 1 min with Triton X-100; this limitation is due to the difficulty of maintaining state 3 respiration for long periods of time. Nevertheless, previous experiments have shown[7] that most, if not all, the solubilizing effect of Triton X-100 takes place in the first minute after adding the detergent to a mitochondrial suspension. On the other hand, we have found that experiments involving shorter detergent treatments are difficult to reproduce.

The qualitative analysis, by SDS-PAGE, of the fractions solubilized by the same amount of Triton X-100 reveals differences between coupled and uncoupled or inhibited submitochondrial particles despite the limited resolution of the gels for such a complex mixture. The possibility of a characteristic lipid composition or enzyme activity distribution in those fractions remains open to investigation.

Our results agree with those of Komor et al.[4] on *Chlorella, Saccharomyces* and *E. coli*, at Triton X-100 concentrations higher than 2×10^{-4} M, in that, in both cases, nonmetabolizing cells or poisoned mitochondria are less affected by detergents than their functionally active, coupled counter-

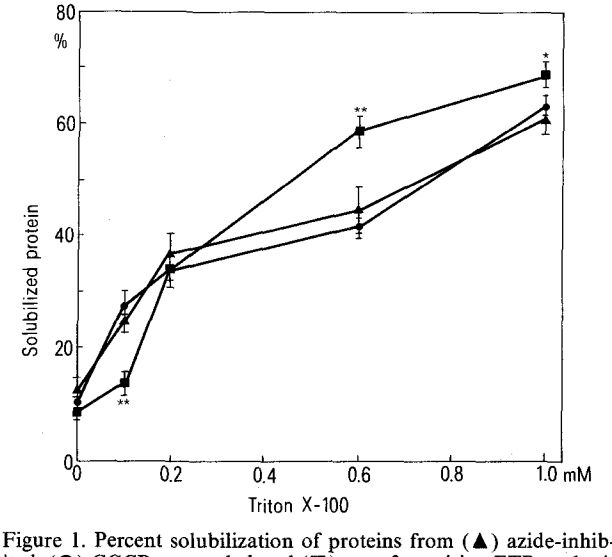

Figure 1. Percent solubilization of proteins from (▲) azide-inhibited, (●) CCCP-uncoupled and (■) state 3 respiring ETP_H submitochondrial particles, as a function of Triton X-100 concentration. Points represent mean values ± SEM (n = 5). Significance (Student's t-test): *$p < 0.05$; **$p < 0.01$.

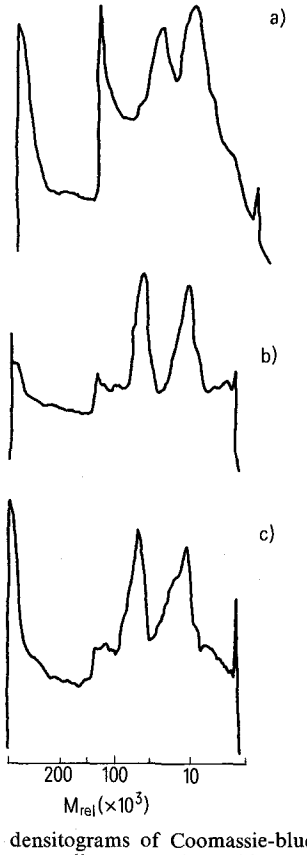

Figure 2. Typical densitograms of Coomassie-blue stained SDS-PAGE gels[16] corresponding to polypeptides solubilized from *a* coupled, *b* uncoupled, and *c* inhibited ETP_H particles by 6×10^{-4} M Triton X-100. All the gels contain the same amount of protein.

parts. The opposite was observed by Lanyi with *Halobacterium cutirrubrum*[3], in accordance with our results at low detergent concentrations. The varying susceptibility of coupled mitochondria, high above 2×10^{-4} M Triton X-100 and low below 2×10^{-4} M detergent, is difficult to explain at the moment.

It is known that conformational changes of the mitochondrial inner membrane accompany its fluctuations in functional state[8], and that these ultrastructural changes determine also changes in molecular conformation, as indicated by studies of accessibility to membrane components[12,17]. These transitions could be invoked in order to explain the different susceptibilities towards Triton X-100; however, no simple explanation is available. State 3 respiring and azide-inhibited mitochondria are indistinguishable from the ultrastructural point of view, both being in the 'condensed' conformation[8]; however, they behave differently towards detergent solubilization. Also, as Triton X-100 is an uncharged molecule, the differences cannot be explained by changes in surface potential. As it is known that there is a selective affinity of Triton X-100 towards certain lipid and protein classes[4,8,9-12], we would like to suggest that the proportion of accessible molecules with high detergent affinity varies with the physiological state of mitochondria, and this determines the qualitative and quantitative differences observed.

1 Acknowledgments: This work was supported in part by a grant from a Spanish 'Comisión Asesora para la Investigación Científica y Técnica'. M.C. Barbero was recipient of a scholarship from the Basque Government.

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0014-4754/84/020193-03$1.50 + 0.20/0

Effect of carbon particles on the recovery of bone marrow stem cells after irradiation in LPS-resistant C3H/HeJ mice[1]

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Summary. Effect of RES-blockade on bone marrow cells was studied serially after irradiation in LPS-resistant mice. Injection of carbon particles reduced damage and accelerated recovery of marrow hemopoietic stem cells, indicating that LPS-resistant mice can react normally to RES-blockade.

Blockade of the reticuloendothelial system (RES) with particulate materials, such as carbon particles (CP) is known to result in increased survival of the irradiated animals[2,3], and this effect has been attributed, at least partly, to its provision of a favorable microenvironment for hemopoietic recovery[4]. Although a similar radioprotective effect is observed in mice pretreated with bacterial lipopolysaccharide (LPS), some differences in the mechanism have been suggested between these two agents. Simultaneous injection of CP and LPS decreases the survival of the irradiated mice[5]. Pretreatment with CP protects the C3H/HeJ mice, which are genetically resistant to most known effects of LPS[6], from radiation lethality, whereas LPS has virtually no effect[7]. We have further demonstrated the difference between the hematological effect of CP and that of LPS on this strain of mice[8]. Since our results also suggested the importance of hemopoietic stem cells in the bone marrow, rather than those in the spleen, in the mechanism of radioprotection, we describe here the recovery of bone marrow stem cells serially after sublethal irradiation in CP-treated C3H/HeJ mice.

Materials and methods. C3H/HeJ mice (Jackson Laboratory, Bar Harbor, USA) were used at 9-10 weeks of age. They were injected i.v. either with 8 mg of CP (Pelican India ink, Gunther-Wagner, Germany) or with pyrogen-free saline 24 h before whole-body irradiation of 450 rad. Irradiation was carried out using a 180 kVp-20 mA X-ray unit with a filter of 1.0 mm Al + 0.5 mm Cu at a dose rate of 50 rad/min. Bone marrow cells were flushed from 4-6 tibias into Fischer's medium, and the total numbers of nucleated cells were counted. The number of granulocyte/macrophage progenitors (GM-CFC) was determined on soft agar by the modified technique of Bradley and Metcalf as previously described[8,9]. Pluripotent stem cells (CFUs) were assayed by the spleen colony method of Till and McCulloch[10]. Briefly, samples of marrow cell suspensions, each from at least 4 mice, were injected into lethally irradiated syngeneic recipients (8 mice per point). 8 days later, spleens were excised under ether anesthesia, fixed in Bouin's solution and the colonies were counted under a dissecting microscope.

Mice used as bone marrow donors were injected i.v. with 5 μg LPS or with 8 mg CP, and marrow cells were harvested 24 h later and injected i.v. into lethally irradiated recipients (1×10^5 cells/mouse). Spleen colonies were counted on day 8 and on day 12 (10 mice/point)

	Number of colonies/spleen	
	On day 8	On day 12
Normal	22.3 ± 1.8	21.5 ± 2.7
LPS*	25.1 ± 2.9	23.4 ± 2.8
CP**	20.8 ± 3.1	22.7 ± 4.2