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Early effects of C_{60} administration in Swiss mice: a preliminary account for *in vivo* C_{60} toxicity.

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Abstract.

High amounts of micronized C_{60} have been injected intraperitoneally into Swiss mice. Until the fourteenth day , they were still alive without any behaviour trouble. C_{60} was well absorbed, and found localized in spleen and liver. Inside the liver, C_{60} was

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detected in Kupffer cells and hepatocytes. But the main result is the induced hypertrophy of the perisinusoidal cells, where C_{60} preferentially accumulates.

Biological properties of C_{60} and its derivatives have not yet been widely studied (1-9). Cultured human leukocytes are able to engulf C_{60} without any acute toxic effect (9), but C_{60} toxicity on living animals is still unknown.

In order to inject high doses of C_{60} (4.0 to 5.0 g per kg body weight), we prepared an aqueous medium containing surface-active and suspending agents and micronized C_{60} (100 mg/ml).

Micronized C_{60} (mC_{60}) (purity 99.9 %), purchased from TechnoCarbo (Plan-de-Grasse, France) is a brown powder composed of aggregates (thickness : 10 to 100 μm) of C_{60} crystals less than 2.0 μm diameter. Micronized C_{60} purity was tested by HPLC, IR, UV and MS.

In a brown glass bottle, 1.0 g of mC_{60} has been added to 7.0 ml of 0.02 % tween 80 (Sigma-Alrich), and 0.9 % NaCl aqueous solution (SA). To this primary suspension, 0.1 g of sodium carboxymethyl-cellulose (CMC) (Sigma-Alrich) has been added and alternatively stirred and sonicated until complete dissolution of CMC was achieved. Then 0.1 g of CMC was added and the final volume was adjusted to 10 ml with solution SA. The resulting homogeneous suspension (SC_{60}) was then sterilized by autoclaving for two hours at 120° C.

This suspension was injected intraperitoneally into 15 specific pathogen free male Swiss mice weighing (20 $\pm$ 2) g and

divided in 3 groups [5 mice per group, injected doses : G_1 (0.5 ml/20 g), G_2 (0.8 ml/20 g), and G_3 (1.0 ml/20 g), respectively]. Three 5-mice control groups (CG_1, CG_2, CG_3) received 0.5 ml/20 g, 0.8 ml/20 g, and 1.0 ml/20 g of suspension system, respectively, in the same conditions as G groups did.

All G_3 mice were still alive until day seven (D_7) when they were sacrified for pathological examination. In the same way, no death was noted in groups G_1 and G_2 which were kept in observation until D_{14} in order to look for any delayed effect due to the suspension system.

Moreover, no difference was observed in the behaviour of G mice as compared to control groups ones. After injection, all animals were stressed for a few minutes. Then only the G_1 and CG_1 mice behave normally. In the groups which received injection volumes higher than 0.5 ml/20 g, the stress period was followed by a decrease of the driving-activity. This could last 8-12 hours for groups G_2 and CG_2 and up to 36 hours for groups G_3 and CG_3. After these times have elapsed, the mice recovered normal behaviour.

At D_7, the average weight of the G_3 mice increased by (49 $\pm$ 5) % as compared with D_0 while group CG_3 average weight increased only by (35 $\pm$ 3) %. This difference has not yet been explained. Anyway, the injected product does not inhibit mice growth.

The absence of mortality and the normal behaviour and growth of the mice prove that the injected C_{60} has neither lethal effect nor acute toxicity in these conditions.

Pathological examination, at D_7 for G_3 and CG_3 mice and at D_{14} for other groups, shows that : i) the livers and the spleens of G mice exhibit the same colour as the injected product ; ii) the bowels of G mice are more coloured than those of control ones ; iii) in the G_2 and the G_3 mice, part of the injected suspension is not absorbed and remains in the vicinity of the injection area, whereas the absorption is complete for the G_1 mice ; iv) the average weights of the livers and the spleens, measured at D_7 for the G_3 mice and expressed in relative percentage with respect to the whole body average weights, increased by 15.5 % and 45.0 %, respectively, as compared to those of CG_3 mice.

These results show that : i) as it was filtered by the spleen and the liver, the injected C_{60} had gone into the systemic circulation ; ii) the C_{60} amounts injected to the mice of G_2 and G_3 exceed their absorption capacity and it is not necessary to further increase doses to demonstrate a possible acute toxicity through this route of administration.

C_{60}-containing accumulations appear as yellow-brown granules brilliantly birefringent when viewed through polarized light under an optical microscope (On the other hand, the suspension system alone barely polarizes light). In the liver, deposits are mainly observed in Kupffer cells and in fat-storing cells (perisinusoidal cells); only few granules were present within hepatocytes. In other tissues such as spleen, lungs, hearts, kidneys, deposits were present in reticuloendothelial cells. No deposit was found in the brain. C_{60} accumulation within fat-

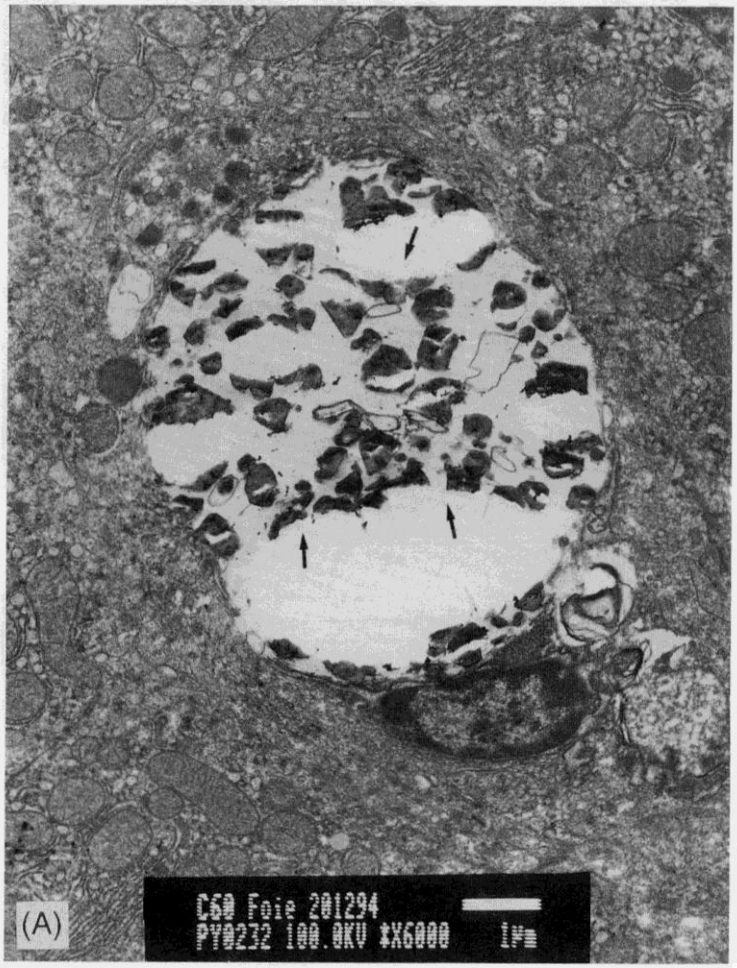

FIG.1. T.E.M. micrographs showing accumulation of C_{60} crystals (arrows) in the liver: A/ inside a fat-storing cell (scale bar = 1 μm), B/ inside a Kupffer cell (scale bar = 1 μm), C/ inside a hepatocyte cytoplasm (scale bar = 1 μm).

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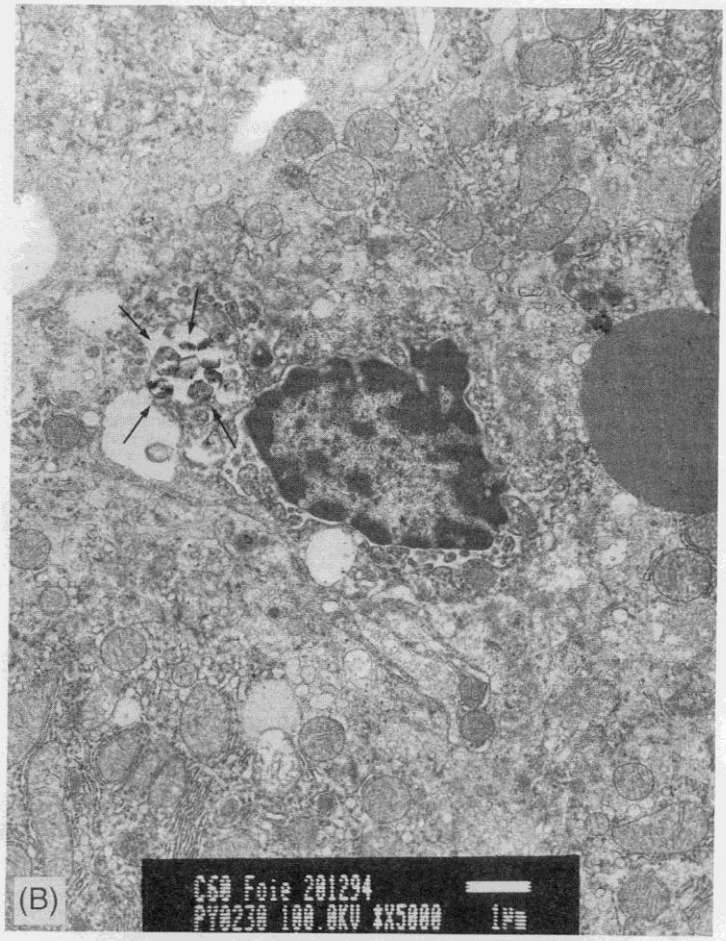

FIG. 1. Continued

storing cells, Kupffer cells and hepatocytes is confirmed by transmission electron microscopy (Fig.1).

The C_{60} accumulation phenomenon in the fat-storing cells discloses a new function of these cells, namely their ability to absorb and accumulate specific solid particles. Indeed, to our

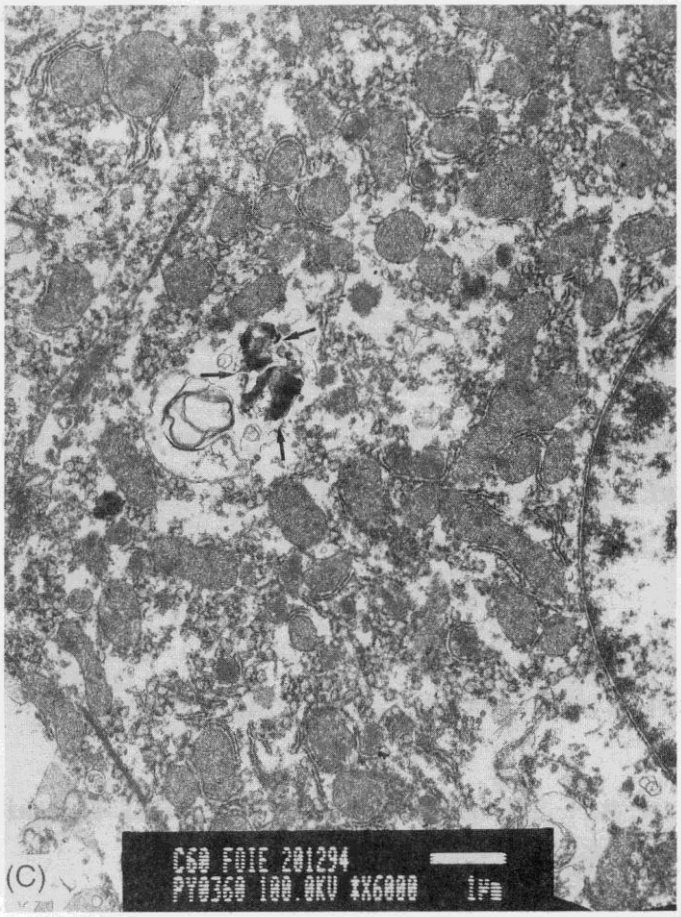

FIG. 1. Continued

knowledge, and as far as the perisinusoidal cells storage functions are concerned, only the retinoid accumulation property was known (10). In the same way, the C_{60} accumulation phenomenon may imply a possible chronic toxicity.

We have now to investigate whether the penetration of C_{60} in hepatocytes is followed by some biotransformation of this

compound and to study the possible emergence of a chronic toxicity.

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