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# INFLUENCE OF DDAB VESICULAR STRUCTURE ON THE γ-RADIOLYSIS OF THE ESTROGEN HORMONE (17α -ETHINYL-ESTRADIOL). RADIATION DAMAGE OF THE AGGREGATES.

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Abstract Using 17α-Ethinyl-Estradiol, a female estrogen hormone, the effect of a typical membrane mimetic agent DDAB (Didodecyldimethylammonium Bromide) on the hormone radiolysis was investigated by competition kinetics in γ-irradiated aggregate systems. The rate constants in the vesicular systems were compared with that in homogenous aqueous solutions. As detected with electron micrography, high radiation doses above 1300 krad induce important non- reversible damage on the vesicular structure.

## INTRODUCTION

In radiolysed systems, the high energy radiation produces ions which are rapidly converted to solvated ions, $e^-_{aq}$, $H_3O^+_{aq}$ and free radicals such as OH and H.

Surfactant vesicles, or liposomes, have been extensively used as biological membrane models.[1]

Radiobiologists are interested in understanding the action of radiation on systems of biological importance such as the biomembranes. Research continues to be very active structural and functional questions, employing models at a molecular level.

We have studied earlier the γ-radiolysis of some estrogen hormones in homogenous aqueous solutions.[2]

In this work, using γ-irradiation and a competition technique, we determined the rate constant for 17α-Ethinyl-Estradiol (E) with OH radicals in a Didodecyldimethylammonium Bromide (DDAB) vesicular system. We also investigated the radiation effect on the vesicular structure using the da-

mage appearing in the electron micrographs after high dose γ-irradiation.

## RESULTS AND DISCUSSION.

We irradiated sonicated, $N_2O$ saturated, DDAB aqueous vesicular systems ([DDAB] = $10^{-2}$ M), at natural pH with various hormone concentrations.

The addition of the appropriate quantity of the hormone was performed by two methods; (a) before sonication of the DDAB solution, (b) after the sonication.

The two series of experiments show no important effect on the OH radical kinetic study.

Fluorescence measurements were not affected by DDAB interference.

We demonstrated, Fig. 1, that there is a linearity between

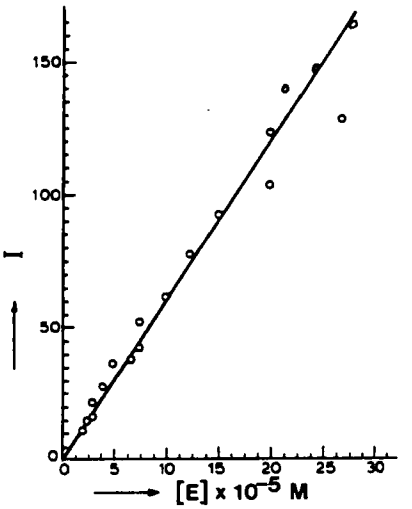


Figure 1. Plot of fluorescence intensity (317 nm) of 17α-Ethinyl-Estradiol in DDAB vesicular systems as a function of its concentration, natural pH ($\lambda_{exc}$ = 294 nm).

the hormone fluorescence intensity and its concentration. Thus, we calculated the Estrogen decomposition G(-E) as a function of the irradiation dose using fluorescence.

During irradiation of $N_2O$ saturated aqueous vesicular

systems, OH is the main radical species present.
The DDAB and the Estrogen hormone compete for OH radicals and the results are given in Fig. 2.

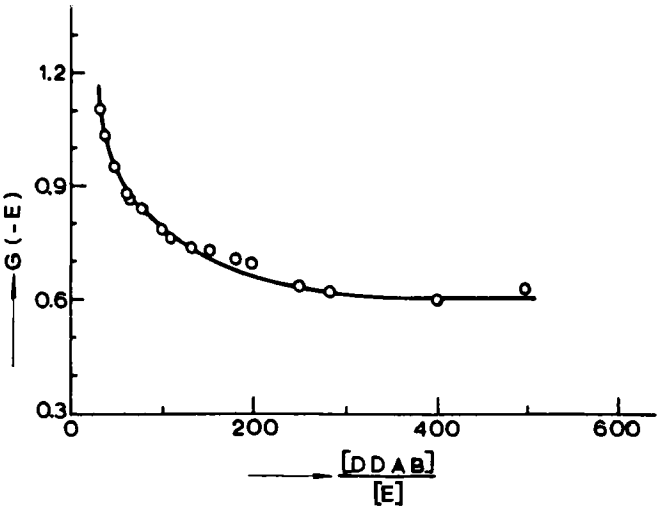


Figure 2. Decomposition yields of 17α-Ethinyl-Estradiol as a function of the concentration ratio of the competitors in DDAB vesicular system. pH, natural, $N_2O$ saturated.

Equation (3) is derived from the competition scheme of equations (1) and (2).

$$OH + DDAB \longrightarrow \text{products} \qquad (1)$$

$$OH + \text{Estrogen} \longrightarrow \text{products} \qquad (2)$$

$$\frac{1}{G(-E)-A} = \frac{1}{G_0(-E)-A} + \frac{1}{G_0(-E)-A}\,\frac{k_{OH+DDAB}[DDAB]}{k_{OH+E}[E]} \qquad (3)$$

$k_{OH+DDAB}$ and $k_{OH+E}$ are the rate constants of reactions 1 and 2 respectively while
[DDAB] and [E] are the concentrations of DDAB and 17α-Ethinyl-Estradiol.
$G_0(-E)$ is the yield of decomposition of 17α-Ethinyl-Estradiol in the absence of DDAB while
A is the extrapolated constant value of G(-E) at "infinite" DDAB concentration, calculated from Fig. 2.
Taking into account the final competition equation (3) and the experimental data of Fig. 2, we obtain Fig. 3.

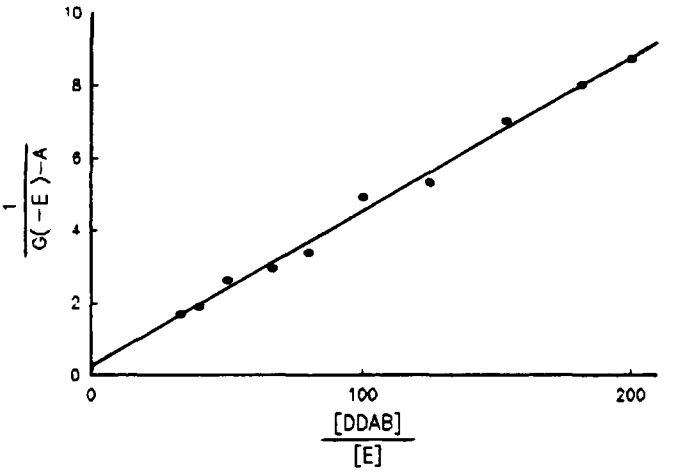


Figure 3. Plot of competition equation (3) as a function of the concentration ratio of the competitors in the vesicular DDAB system. pH=natural, $N_2O$ saturated.

From this figure and taking also into account that $k_{OH+DDAB}$ = 5.67 x $10^9$ $M^{-1}$ $s^{-1}$,[3] we calculated $k_{OH+E}$ = 3.61 x $10^9$ $M^{-1}$ $s^{-1}$.

The experimental points of Fig. 2 were obtained from vesicular systems where the hormone was added after sample sonication.

The same experimental procedure was followed for samples where the hormone was present during the sonication and a rate constant $k_{OH+E}$ = 2.28 x $10^9$ $M^{-1}$ $s^{-1}$ was calculated, which is very similar with the previously determined value.

After comparison of these two rate constants with that found in homogenous aqueous solutions ($k_{OH+E}$ = 5.98 x $10^9$ $M^{-1}$ $s^{-1}$)[4], we can conclude that there is no important effect on the rate constants due to the vesicular system irrespective of the preparation procedure.

Finally, we studied the damage produced by γ-radiation on the form of the DDAB vesicles. We irradiated samples of the DDAB sonicated vesicular system, $N_2O$ saturated, with doses of 1360 krad. After an appropriate preparation of the sample (staining, drying under appropriate conditions, etc.)

electron micrographs were taken, Fig. 4a. After comparison

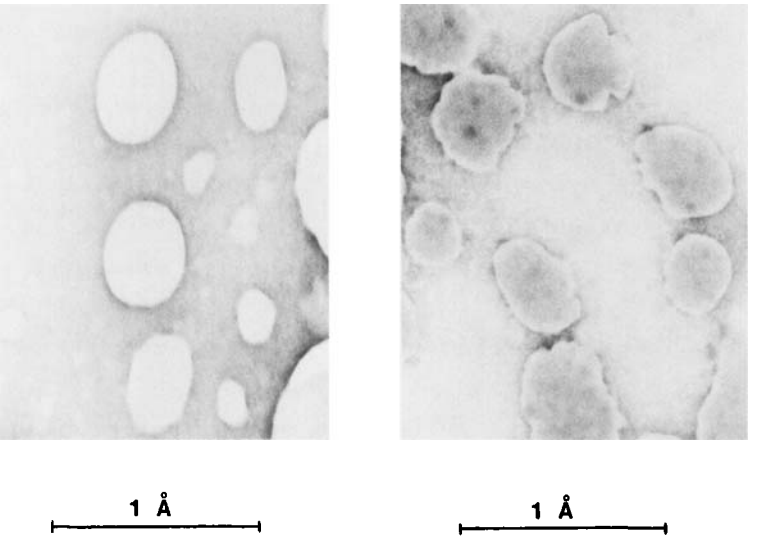


Figure 4. Electron micrograph of vesicular aggregates (using uranyl acetate as dye) (a) irradiated with 1360 krad ($N_2O$ saturated) and (b) unirradiated.

with micrographs of unirradiated samples, Fig. 4b, we can conclude that there is an obvious modification of the morphology of the irradiated vesicles, without important changes of their size.

The irregularities (cavities) in the polar surface are the result of the damage produced by the reaction of OH and H radicals on the DDAB molecules. Usually the abstraction of an H atom from a C-H bond, the C-C bond rupture and the reaction with the counterions of the vesicular structure are the causes of alteration of the intra-molecular forces. This is the case for Fig. 4b where water molecules enter the bilayer producing anomalies in the smecticity of the vesicles.

## EXPERIMENTAL

Didodecyldimethylammonium Bromide (DDAB) was prepared and purified as described elsewhere.[5,6]

17a-Ethinyl-Estradiol (E), was obtained from Merck, bio-

chemical purity.

Vesicles were prepared by the sonication method (MSE sonicator, 30 min) and then extruded through 0.2 μm cellulose nitrate filters (Sartorius).

17α-Ethinyl-Estradiol was added (a) before or (b) after the sonication of DDAB solutions. In both cases the Estrogen-DDAB vesicular system was incubated for 20 h at 44° C.

Before irradiation all samples were deaerated by flushing with $N_2O$, scrubbed with $V^{2+}$. Triply distilled water was used in all cases.

Irradiations were carried out in a $^{60}Co$ Gammacell 200 at a dose rate of ca. 450 rad $min^{-1}$.

Hydroxyl radical reactivity data were obtained by a competition technique, where DDAB and 17α-Ethinyl-Estradiol compete for OH radicals. The Estrogen decomposition was followed from measurements of its fluorescence intensity at 317 nm ($\lambda_{exc}$ = 284 nm).

The absorption spectra were recorded on a Perkin-Elmer 551 spectrophotometer, while fluorescence spectra on an Aminco-Bowman spectrophotofluorometer.

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