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CONTROL OF FATTY ACID INCORPORATION IN MEMBRANE PHOSPHOLIPIDS

X-RAY-INDUCED CHANGES IN FATTY ACID UPTAKE BY TUMOR CELLS

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Lymphosarcoma cells isolated from the spleens of tumor-bearing mice were used to study the effect of a low dose of X-rays (5 Gy) on the incorporation of [^{3}H]palmitate and [^{14}C]arachidonate into the lipids of the tumor cells. Palmitate and arachidonate were rapidly incorporated especially into the phospholipids of the cells. Between one and three hours after the start of the incubation with radiactive palmitate 80–90% of the label of the total lipids was found in the phospholipid fraction. Already after a few minutes of incubation with radioactive arachidonate, about 95% of the label was incorporated in the phospholipids. Irradiation caused a small but significant increase in the rate of fatty acid incorporation for both fatty acids. Concomitantly, a significantly increased amount of fatty acid was removed from the medium by the cells as a result of the irradiation, and the specific radioactivity of the free fatty acids in the cells was found to be enhanced. The radiation effect on the tumor cells could be mimicked by a hypotonic treatment. The magnitude of the radiation-induced stimulation of the fatty acid incorporation was similar to that of the hypotonically induced effect. Cells which had received a hypotonic treatment before the irradiation, did not show an additional radiation-induced enhancement of fatty acid incorporation into the cellular lipids. When the cells were incubated with serum albumin loaded with a relatively large (non-physiological) amount of complexed fatty acids (fatty acid: albumin molar ratio, $\bar{\nu} = 3.7$), no radiation effect on the fatty acid incorporation could be detected. It is concluded that hypotonic treatment, irradiation, and increased supply of exogenous fatty acids all lead to an enhanced flux of fatty acids into the cells. These results confirm our previous suggestion that the uptake of fatty acids through the plasma membrane is the rate-limiting step in the fatty acid incorporation into the phospholipids and that ionizing radiation is one of the means to enhance fatty acid uptake through the plasma membrane leading to an increased incorporation into the phospholipids.

Introduction

In a previous paper [1] we showed that the incorporation of labelled fatty acids into the phospholipids of subfractions of tumor cells in vivo was enhanced after low doses (2.5–5 Gy) of X-rays. These studies were performed with lymphosarcoma-bearing mice which had been total-body irradiated. The results described were suggestive of an enhanced uptake of fatty acids by the tumor cells after irradiation. To exclude indirect effects as caused by irradiation of other organs of the animal, it was decided to pursue this problem by studying fatty acid incorporation into the lipids of

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the isolated tumor cells in vitro. Such an approach offers the possibility to manipulate the incubation conditions in ways, which are difficult to achieve in vivo, where the incubation medium by necessity is whole blood.

The mechanism of fatty acid uptake by cells has not been studied extensively. A passive diffusion is thought to play a major role [2,3] although supplementary and alternative ways of uptake have been suggested [4–6]. Some reports [7–10] suggest that under normal physiological conditions fatty acid uptake can be the limiting step in phospholipid synthesis.

The results of this investigation indicate that X-irradiation enhances plasma membrane permeability of the tumor cells for fatty acids and lead us to conclude that fatty acid uptake is the rate-limiting step in the incorporation of fatty acids into the phospholipids of the tumor cells.

Materials and Methods

Animals; tumor cells. Male C57bl mice with transplanted lymphosarcoma tumor were used. Details of the tumor system were presented in a previous paper [1]. The tumor spleen, weighing about 600 mg, was extirpated, minced with scissors and gently pressed through two layers of nylon gauze with the aid of 8–10 ml balanced salt solution (BSS, consisting of 4.5 mM D-glucose, 14 mM NaCl, 45 μM $CaCl_2$, 0.88 mM $MgCl_2$, 4.9 mM KCl and 130 mM Tris-HCl (pH = 7.6)). The cells in the filtrate were gently squeezed through the needle (0.9 × 38 mm) of a syringe (20 ml) in order to dissociate small cell aggregates. This procedure was repeated once. The suspension of cells was layered on a cushion of 10 ml Lymphoprep (Nyegaard, Norway) in a 40 ml cellulose nitrate tube and centrifuged for 20 min at 1000 × *g*. The tumor cells were collected from the layer on top of the lymphoprep with a Pasteur pipette, washed twice in 40 ml balanced salt solution and suspended in the appropriate medium. Generally the cells were suspended in RPMI culture medium (1640, Flow Laboratories, Scotland, U.K.) supplemented with 5 ot 10% fetal calf serum (629, Gibco Bio-Cult., Scotland, U.K.). The cells were counted in a Bürker Chamber in 0.1% Trypan blue and adjusted to a suitable concentration (usually 0.64 · 10^7 cells/ml). All cell handling was done at ambient temperature.

Irradiation. The cell suspension was divided in portions of 10 ml and irradiated in erlenmeyer flasks (25 ml) at 20°C or 37°C. The Philips-Müller MG-300 X-ray machine was operated at 200 kV and 15 mA. The beam was filtered with 0.5 mm Cu and 0.5 mm Al; the half-value layer (HVL) was 1.1 mm Cu. The focus sample distance was 51 cm and the dose 0.6 Gy/min. After irradiation the cells were either directly prepared for lipid extraction or incubated in a shaking waterbath (100 rpm) at 37°C.

Incubation and further handling of the cells. Incorporation of fatty acids was studied by adding a 0.5 ml solution of radioactive palmitate or arachidonate as tracer substances to a 10-ml cell suspension. The fatty acids were complexed to bovine serum albumin as described before [1]. In a volume of 0.5 ml the following tracers were added: 0.1–2.0 μCi of [9,10(n)-^{3}H]palmitic acid (RCA, Amersham, U.K., CFB-37), [1-^{14}C]palmitic acid (RCA, TRA-21) or [1-^{14}C]arachidonic acid (RCA, CFA-504) either separately or simultaneously. The flasks were incubated for the times indicated at 37°C in a shaking water bath (100 rpm). At the end of the incubation period, the suspensions were transferred to a 50-ml centrifuge tube and rapidly cooled in ice. The cells were pelleted by centrifugation at 4°C at 900 × *g* for 5 minutes. For those experiments in which total radioactivity in the incubation medium had to be determined, 1-ml aliquots of the supernatant were transferred to glass scintillation vials and mixed with 10 ml Pico-Fluor-30 and the radioactivity was measured as described before [1]. The cell pellet was resuspended in 30 ml cold saline (0.9% NaCl) and centrifuged again. The cell pellet was suspended in 4 ml of saline and total lipids were extracted as described before [1].

In some experiments a tracer amout, 3 μCi of [1(3)-^{14}C]glycerol (RCA, CFA-47), was added to the incubation medium together with the albumin-complexed radioactive fatty acids. Incubation and lipid extractions were done as described above.

Permeability studies with sucrose were performed in 10 mM sucrose (Merck, 7651 E) labelled with 0.27 μCi [U-^{14}C]sucrose (RCA, CFB-146).

The cells were incubated in 10.5 ml RPMI medium supplemented with 10% fetal calf serum. After the incubation period the cells were washed twice with a buffer solution (0.25 M sucrose, 50 mM Tris-HCl (pH = 7.4), 2.5 mM KCl and 5 mM $MgCl_2$). The cell pellet was lysed by adding 2 ml of a 2% (v/v) solution of Triton X-100 (Sigma, U.S.A.) in distilled water. The lysate was assayed for radioactivity after adding 10 ml Pico-Fluor in a glass counting vial.

For experiments in which two different fatty acid: albumin ratios were used, the incubation media were prepared as follows. To make up the high-ratio medium 0.5 ml of fetal calf serum, (which contains about 0.22 μmol serum albumin [11], which is 15.4 mg assuming a molecular weight of 69000, and 0.12 μmol free fatty acid [12]), was added to 10 ml cell suspension in RPMI medium, followed by 1 ml of a solution containing 4.1 μCi of [^{3}H]palmitate and 2.1 μmol palmitate complexed to 25 mg (0.36 μmol) bovine serum albumin (fatty acid free). This resulted in an albumin content of 40.4 mg (0.59 μmol) in a total volume of 11.5 ml, and final fatty acid: albumin ratio of $\bar{\nu} = 3.7$ (2.2 μmol fatty acid and 0.59 μmol albumin). The low-ratio medium was prepared similarly, i.e. in 11.5 ml of the same medium, 0.41 μmol fatty acid was complexed to 0.59 μmol albumin resulting in a final fatty acid: albumin ratio of $\bar{\nu} = 0.7$. In the two experiments the specific radioactivities of the fatty acids were the same.

Details for each experiment are given in the legends to the figures.

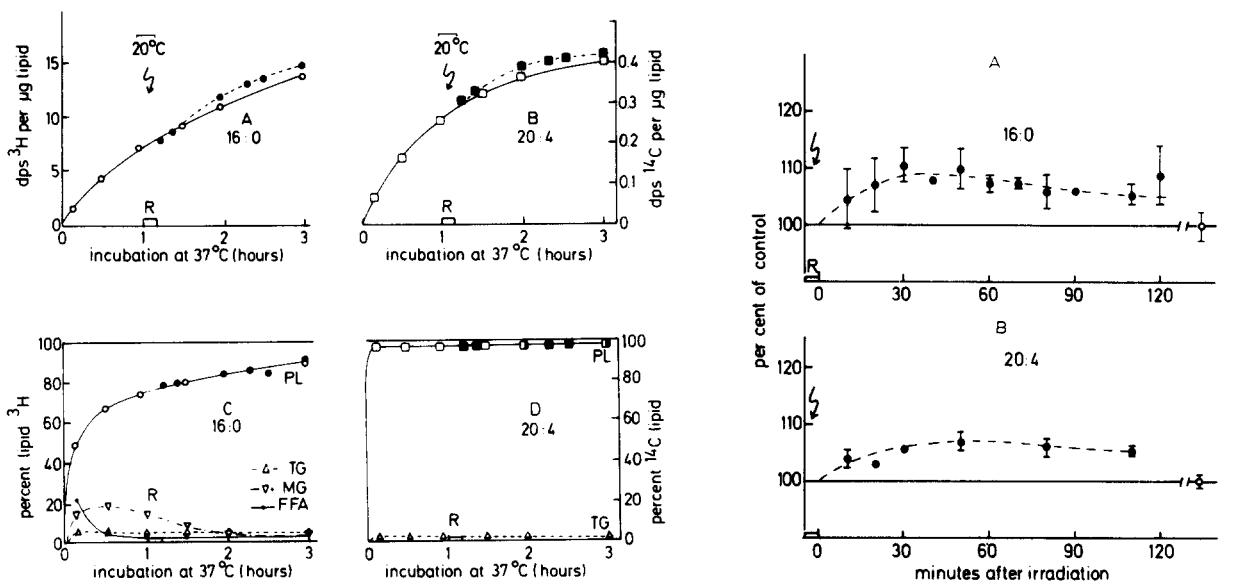

Fig. 1. The effect of X-irradiation on fatty acid incorporation into the lipids of lymphosarcoma cells. To 20 ml of a suspension of isolated lymphosarcoma cells ($1.28 \cdot 10^7$ cells/ml) in RPMI medium containing 10% fetal calf serum, 0.5 ml of radioactive tracer fatty acids complexed to bovine serum albumin was added. For this experiment 1.4 μCi [^{3}H]palmitate and 0.4 μCi [^{14}C]arachidonate were used simultaneously. The incubation was performed at 37°C as described in the methods. After 1 h incubation some of the flasks were irradiated with a dose of 5 Gy at 20°C and reincubated at 37°C. Incubations were stopped at different times and lipids were extracted from the cells as described in the methods. Open symbols (○) are control conditions while solid symbols (●) illustrate irradiation conditions. (A) Incorporation of [^{3}H]palmitate into the total lipid fraction of the cells. (B) Incorporation of [^{14}C]arachidonate. (C) The distribution of [^{3}H]label derived from palmitate. (D) The distribution of [^{14}C]label derived from arachidonate. PL, phospholipids; TG, triacylglycerols; MG, monoacylglycerols; FFA, free fatty acids.

Fig. 2. The effect of X-irradiation on fatty acid incorporation. The data represent a collection of independent experiments. (A) Incorporation of [^{3}H]palmitate, nine experiments. (B) Incorporation of [^{14}C]arachidonate, two experiments. In all experiments the cells were irradiated with 5 Gy of X-rays 1 h after the start of the incubation. Open symbols (○) are controls, while solid symbols (●) illustrate the irradiated condition. The error bars represent the standard deviation of the mean.

Results

Effect of X-irradiation on fatty acid incorporation into neutral and phospholipids

Lymphosarcoma cells were isolated from the spleens of tumor-bearing mice and incubated in RPMI medium with fetal calf serum and radioactive fatty acids complexed to lipid-free bovine serum albumin. In a first set of experiments [^{3}H]palmitate and [^{14}C]arachidonate were simultaneously present in the incubation medium consisting of RPMI and 10% fetal calf serum. The incorporation of the fatty acid label into the cellular lipids was followed during 3 h. The cells were X-irradiated one hour after the start of the incubation. A typical result of such an expermient is presented in Fig. 1. A small but significant (see also Fig. 2) radiation-induced increase of incorporated radioactive label for both fatty acids in the total lipid fraction was observed. The distribution of the labels among different lipid classes is shown in Fig. 1C and Fig. 1D. The [^{3}H]palmitate label is predominantly found in the phospholipids. After 30 min this is more than 70%, increasing up to 90% after 3 h of incubation. Of the neutral lipids the monoacylglycerols are the most heavily labelled components. A maximum of label content in this lipid (20%) is seen after 30 min of incubation. The diacylglycerols and triacylglycerols appear to be only marginally labelled under these conditions. Of the [^{14}C]arachidonate label more than 95% is found in the phospholipids within 10 min after the start of the incubation (Fig. 1D). A very low fraction of the ^{14}C-label is present in the triacylglycerols. Similar to the in vivo situation [1], there was no change in the distribution of the label among the lipids in the in vitro experiments as a results of the irradiation (Fig. 1C and 1D), i.e. nearly all incorporated label was in the phospholipid fraction. This indicate that the increased fatty acid incorporation into the total lipids (Figs. 1A and 1B) is due to an increase in specific radioactivity in the phospholipid fraction (see also Ref. 1).

The radiation effect may seem small, but the statistical significance of the observed increase in specific radioactivity is evident, and is illustrated

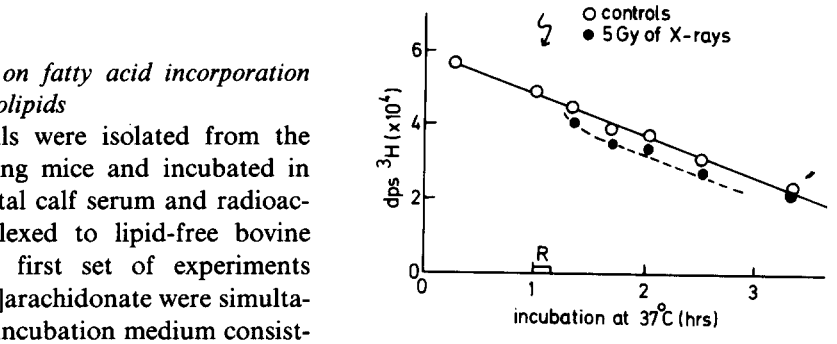

Fig. 4. The effect of X-irradiation on the content of radioactive label in the medium of the suspended lymphosarcoma cells. Conditions as described in Fig. 1. [^{3}H]Palmitate was used as the fatty acid tracer. Open symbols (O), controls; solid symbols (●), irradiated conditions.

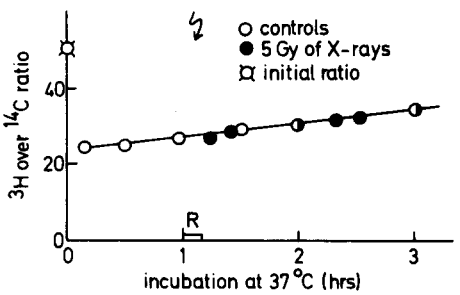

Fig. 3. The effect of X-irradiation on the ^{3}H/^{14}C ratio of total lipids after incubation of tumor cells with [^{3}H]palmitate and [^{14}C]arachidonate. Open symbols (O), controls and closed symbols (●), irradiated conditions. The ^{3}H/^{14}C ratio of the fatty acids in the medium at the start of the incubation is indicated at zero time. Conditions as in Fig. 1.

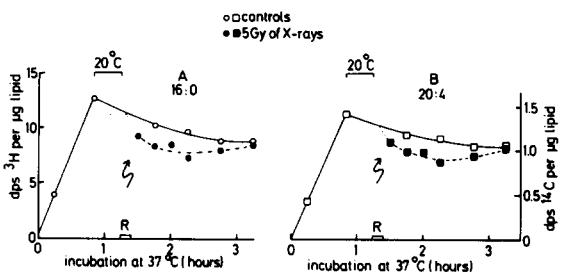

Fig. 5. The effect of X-irradiation on the specific radioactivity of lipids of lymphosarcoma cells pre-incubated with [^{3}H]palmitate and [^{14}C]arachidonate. Pre-incubation was as in Fig. 1 except that $0.64 \cdot 10^7$ cells/ml and 2.1 µCi [^{3}H]palmitate and 0.1 µCi [^{14}C]arachidonate were used. At 50 minutes after the start of the incubation the medium was replaced by label-free medium. (A) Preincubation with [^{3}H]palmitate. (B) Pre-incubation with [^{14}C]arachidonate.

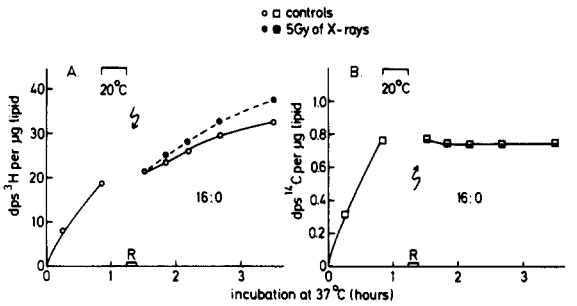

Fig. 6. Effect of X-irradiation on the specific radioactivities of the lipids in lymphosarcoma cells, pre-labelled with 1.8 µCi [^{3}H]palmitate (A) and 0.18 µCi [^{14}C]palmitate (B) and reincubated in a medium in which only [^{3}H]palmitate was present in tracer amounts. The pre-irradiation conditions were as in Fig. 5, except for the tracers. The post-irradiation conditions were without fetal calf serum but with 45 mg bovine serum albumin (fatty acid free) and 1.8 µCi of [^{3}H]palmitate complexed to it as a radioactive tracer.

in Fig. 2, which summarizes the results of nine different experiments for palmitate and of two experiments for arachidonate. The increase in specific radioactivity observed in the irradiated cells lasted for at least 2 h. The ratio of incorporation of ^{3}H- and ^{14}C-label in the lipids was the same for irradiated and control cells (Fig. 3), which is consistent with our previous findings after irradiation of tumor cells in vivo [1].

Uptake of fatty acid, glycerol and sucrose

From the in vivo experiments [1], it was suggested that the uptake of fatty acids by the cells was increased after irradiation. To investigate this possibility in vitro, the depletion of label from the medium was measured under incubation conditions similar to those presented in Figs. 1 and 2. The tumor cells were incubated with [^{3}H]palmitate. The label remaining in the medium rapidly decreased over a period of several hours (Fig. 4). As can be seen, there was a significant increase in the amount of label removed from the medium as a result of irradiation. More than 95% of the ^{3}H-label in the medium was identified as free fatty acid, even after 3 h of incubation (not shown).

These experiments indicate a radiation-induced enhancement of fatty acid uptake from the medium by tumor cells. To further explore this possibility the following experiments were performed. Cells were incubated in RPMI medium with fetal calf serum and [^{3}H]palmitate plus [^{14}C]arachidonate. After 50 min of incubation the cells were pelleted and resuspended in fresh medium with fetal calf serum but without radioactive tracers. The cells were X-irradiated immediately after changing the incubation medium, such that both irradiation and

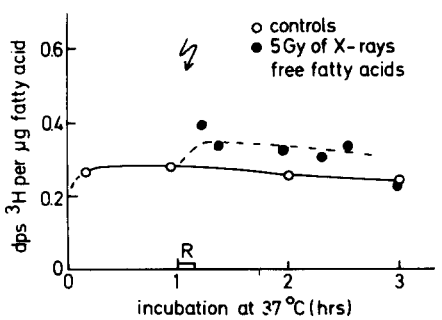

Fig. 7. The effect of X-irradiation on the specific radioactivity of the free fatty acid fraction in the lymphosarcoma cells after incubation with 1.4 µCi [^{3}H]palmitate. Cells were incubated under conditions as given in Fig. 1.

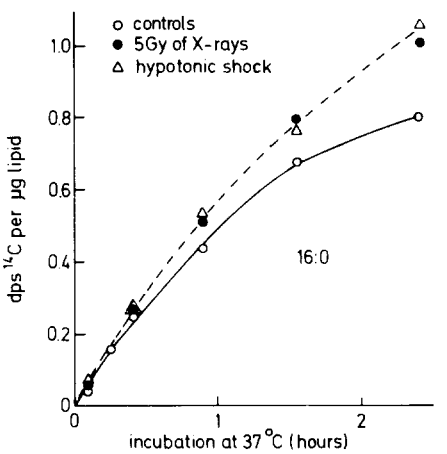

Fig. 8. Effects of hypotonic shock or X-irradiation on the specific radioactivities of the total lipids of lymphosarcoma cells, incubated with [^{14}C]palmitate. Cell suspensions were X-irradiated with a dose of 5 Gy at 20°C, or given a hypotonic shock, 22 min before the start of the incubation. The hypotonic shock was performed by dilution of the cell suspension (10 ml) 2-fold with distilled water followed by incubation at 37°C for 5 min. Hereafter all suspensions were supplemented with fresh RPMI-medium plus 10% fetal calf serum. At the start of the incubation at 37°C, 0.5 ml of a trace amount of [^{14}C]palmitate complexed to 5 mg fatty acid free bovine serum albumin was added to the cell suspensions. For each incubation 0.09 µCi [^{14}C]palmitate was used. Conditions as described in the legend to Fig. 1.

the subsequent incubation were done in absence of radioactive fatty acid tracer in the medium. The specific radioactivity of the total lipids was determined at different times before and after irradiation. The results of these experiments are shown in Fig. 5. A decreasing amount of label is found in the lipids of the cells as the incubation proceeds, and X-irradiation accelerates this decrease in label content. The radiation effect is similar for palmitate and arachidonate.

The accelerated decrease in specific radioactivity in the lipids may result from a dilution effect caused by enhanced entrance of unlabelled fatty acids from the medium (fetal calf serum) or from leakage of endogenous fatty acids out of the cells. In order to distinguish between these two possibilities the cells were incubated in fatty-acid-free medium with only tracer amounts of fatty acids (Fig. 6). The incubation was done as in the foregoing experiment except that during and after irradiation the incubation was continued in RPMI-medium without fetal calf serum and in presence of only tracer amounts of [^{3}H]palmitate complexed to 45 mg (0.65 μmol) fatty-acid-free bovine serum albumin. As can be seen in Fig. 6A, the expected increase of the specific radioactivity in the tritium-labelled lipids could be detected, but no change in specific radioactivity in the ^{14}C-label was found after irradiation (Fig. 6B). This experiment suggests that the decrease in specific radioactivity found in Fig. 5 does not result from radiation-induced leakage of fatty acids from the cells.

The increased entry of radioactive fatty acids into the cells during and after irradiation is reflected in a raise in specific radioactivity of the free fatty acids in the irradiated cells (Fig. 7).

Glycerol is also used by the cell for the synthesis of acylglycerols and phospholipids. A number of experiments were performed in which [^{3}H]palmitate and [^{14}C]glycerol were simultaneously incubated with the cells. No increase in [^{14}C]glycerol label was found after irradiation, while the effect on fatty acid incorporation was again apparent (not shown). Also the uptake of [^{14}C]sucrose was measured. In contrast to glycerol this is not metabolized. Only a slow rate of entrance of sucrose was detected during the incubation, probably mediated by pinocytosis. Only 0.04% of the total available amount of sucrose in the medium was found associated with the washed cells after 3 h of incubation. X-irradiation at one hour after the start of the incubation did not have any effect on the recovery of radioactivity in the tumor cells.

Fatty acid incorporation in hypotonically treated cells

The results of the experiments described above suggest that the increased incorporation of the fatty acids into the lipids after X-irradiation is a result of an increased intracellular availability of fatty acids. To obtain further proof of this supposition we decided to investigate if other methods to increase membrane permeability would also enhance fatty acid incorporation. Fig. 8 shows that hypotonic shock mimics the radiation effect. Both treatments resulted in about the same degree of stimulation of fatty acid incorporation in the lipids of the lymphosarcoma cells.

It may very well be that either one of those conditions leads to a situation in which the fatty acid supply is no longer rate limiting for the lipid synthesis. If this is true, no radiation effect is to be expected when the cells receive hypotonic treatment prior to the X-irradiation. The incorporation of palmitate and arachidonate are not influenced by irradiation when hypotonically shocked cells are used (not shown).

The influence of the molar ratio of fatty acid to albumin

Except from the experiments described in Fig. 6 all incubations were done in presence of fetal calf serum. As suggested in the foregoing experiments, the intracellular availability of fatty acids (as substrates for lipid synthesis) is limited by the amount of fatty acids present in serum. The molar ratio of fatty acid: albumin ($\bar{\nu}$) in these preparations was of the order of 0.5–1.0. The fatty acid to albumin ratio in plasma is of the same magnitude [13]. This ratio might be one of the parameters regulating the amount of free fatty acids in the cytosol [13,14].

In a number of experiments incubations were done with lymphosarcoma cells in media with a constant amount of albumin, to which different amounts of free fatty acids were complexed with a fixed specific radioactivity. In Fig. 9 the results are shown of radiation experiments on cells incubated

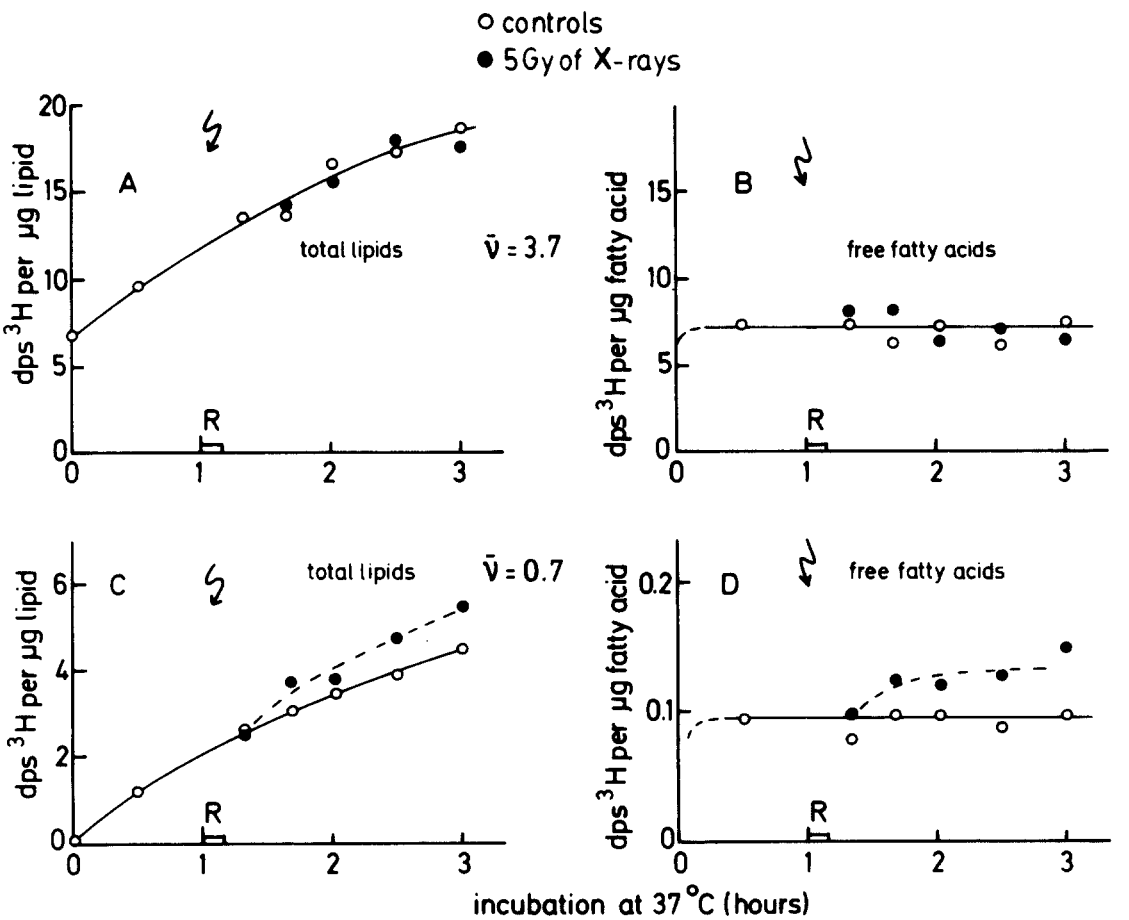

Fig. 9. Effect of X-irradiation on the specific radioactivities of total lipids (A and C) and free fatty acids (B and D) of lymphosarcoma cells incubated with a high and a normal free fatty acid to albumin ratio. The incubation medium consisted of a high (A and B) molar ratio of fatty acids to albumin ($\bar{\nu}$ = 3.7), or a lower (C and D) ratio ($\bar{\nu}$ = 0.7). Solid symbols (●) represent X-irradiated cells, open symbols (○) are controls. Details of the preparation and incubation conditions are given in Methods.

with albumin heavily loaded with fatty acids ($\bar{\nu}$ = 3.7). No effect of radiation on the incorporation of fatty acids in the lipids (Fig. 9A) could be observed under these conditions, nor was there a change in the specific radioactivity of the intracellular fatty acids (Fig. 9B). When the ratio of fatty acid to albumin was lowered to physiological values ($\bar{\nu}$ = 0.7), the radiation effect was clearly present. A higher incorporation of palmitic acid into the lipids (Fig. 9C) corresponded again with a higher specific radioactivity of the fatty acids inside the cells (Fig. 9D). All experiments illustrated in Fig. 9 were done with pooled cells derived from one group of animals and the incubation and radiation conditions were the same except for the difference in $\bar{\nu}$.

Discussion

In a previous paper [1] it was shown that total body irradiation of lymphosarcoma-bearing mice resulted in an enhanced fatty acid incorporation into the lipids of the tumor cells. The experiments with isolated lymphosarcoma cells, presented in this report, show that the radiation effect was also present when the tumor cells were irradiated in vitro.

The fatty acids were rapidly incorporated into

the lipids of the tumor cells. The results were grossly compatible with the in vivo incorporation studies [1] except for some minor differences. Monoacylglycerols were found to take up a higher proportion of label during in vitro incubation with [^{3}H]palmitate at the expense of triacylglycerols and the incorporation of [^{14}C]arachidonate label into the phospholipids was more pronounced under the in vitro conditions. Finally, the radiation effect was somewhat less pronounced than in the in vivo system but lasted longer.

The results with glycerol and sucrose suggest that the radiation-induced change in the permeability of the plasma membrane as we found it for fatty acids, is not a general phenomenon.

From the experiments in this and in the previous paper [1], we tend to conclude that the incorporation of fatty acids into the phospholipids of the tumor cells in vivo is limited by the transport from the blood plasma across the plasma membranes.

When the tumor cells were irradiated after hypotonic treatment or when they were irradiated in a medium with a high fatty acid : albumin ratio (Fig. 9) no enhanced fatty acid incorporation into the cellular lipids was observed. Apparently, under these conditions fatty acid supply is no longer rate limiting for phospholipid synthesis. This is compatible with the work of Oram et al. [8] who showed with perfused rat hearts, that when the fatty acid to albumin ratio ($\bar{r}$) was higher than 1.4, the available amounts of intracellular fatty acids appeared to be sufficient to attain maximal lipid synthesis. The fact that no radiation effect is seen under conditions of increased fatty acid supply, indicates that low-dose irradiation does not interfere with rate-limiting enzymes of phospholipid synthesis, including those that are responsible for the turnover of acyl groups.

Fig. 9 shows that an approximately 5-fold increase in the fatty acid : albumin ratio, and thus in the absolute fatty acid concentrations in the incubation medium (A and C), leads to a much higher increase in the specific radioactivities of the intracellular free fatty acids (B and D). Although this may seem remarkable, earlier studies with Ehrlich ascites cells [7,13,15] have shown that intracellular fatty acid levels can increase disproportionally with increasing fatty acid : albumin ratios in the medium. In addition, passive adsorption of fatty acids to the cell surface may contribute to the observed high increase in intracellular specific fatty acid radioactivity. The high zero-time value of the specific radioactivity of the total lipids as seen in Fig. 9 is in line with this view. The marked increase in the specific radioactivity of intracellular fatty acids (Figs. 9B and 9D) was not reflected in a corresponding increase in specific radioactivity of the total lipids (Figs. 9A and 9C). This is in agreement with the results of Spector and Steinberg [13], who found that at higher fatty acid : albumin ratios the increase in fatty acid concentration in the cells was not proportional with either fatty acid oxidation or esterification.

Although the evidence presented in this and the previous paper [1] supports the idea that radiation alters the rate of entrance into the cells of free fatty acids from the medium, it is not clear what type of membrane alterations are underlying this phenomenon. It has been suggested that changes in membrane permeability in erythrocytes as caused by radiation might be the result of lipid peroxidation [16]. Our results [1,17,18] do not support this view; at low doses of radiation no indication of lipid peroxidation could be observed. Yonei et al. [19] have shown conformational changes of membrane proteins at a dose of 1 Gy. At this dose he also reported [20] an increase in lipid fluidity of the membrane. The change in lipid fluidity might be an indirect effect caused by an altered protein-lipid interaction.

The molecular mechanism involved in the radiation damage of the plasma membrane is currently under investigation in our laboratory.

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