# THE RELATION BETWEEN THE CHEMICAL STRUCTURE AND BIOCHEMICAL PROPERTIES OF UNSATURATED FATTY ACIDS IRRADIATED WITH THE X-RAY

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# X線照射不飽和脂肪酸における化学構造と生化学的性状との関係

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不飽和脂肪酸を乳化状態としてX線照射し照射脂肪酸の化学的変化を自働酸化の面より追求し同時にその生物作用を検討した．その結果照射脂肪酸が酸化に対して不安定であり二重結合の移動，過酸化物の生成，カルボニルの生成度合が大きく，かような変化の著しいものほど細胞のコハク酸脱水素酵素活性の低下，ラット肝ミトコンドリアの膨潤作用も強いことが判明した．かゝる現象を既知構造物質（不飽和度，二重結合の共軛度，立体構造，官能基等の異った既知物）について検討を行った結果，酸化の attack をうけやすい脂肪酸及びその誘導体ほど細胞膜変性の度合が高く又ミトコンドリアの酸化的燐酸化の阻害作用も大であった．又リノール酸，オレイン酸についてそれらのナトリウム塩，カリウム塩，低級アルコールエステル，モノグリセリド，トリグリセリド（リノール酸系列のものとしてサフラワー油，オレイン酸系列のものとしてオリーブ油を使用した）の如きacyl 基結合の形態を異にしたものについて生物作用を比較した結果，脂肪酸のアルカリ塩，遊離脂肪酸，モノグリセリド，エステル類，トリグリセリドの順に活性が強く，同一結合型については親水性の大きいしかも分子量の小さいものの方が細胞膜透過性の度合を高め，又ミトコンドリアの酸化的燐酸化の阻害度も高いことが明らかになった．

The uncoupling of oxidative phosphorylation and hemolysis are the typical effects of X-ray irradiation on animal. The auther found that the lipid fraction of X-ray irradiated rabbit organs had the uncoupling factor which showed the strong hemolytic action[1]. These actions can be seen in the lipid fraction of normal rabbit liver, but that of X-irradiated rabbit liver shows the more strong uncoupling action. Moreover, this uncoupling factor has the inhibitory action to the growth of HeLa cells[2]. Namely, the change of lipid metabolism with X-ray irradiation is one of the most important biological actions of X-ray irradiation. Related subject to the action of X-ray irradiation on the lipid metabolism of living cells' it was studied by many authers and was reviewd recently by Chevallier[3]. These authers noticed an accelerated lipid metabolism, an increase of fatty acid in the blood and an induced hyperlipemia[4),5]. Against these indirect actions of X-ray irradiation on the animals, a direct action of X-ray irradiation on the lipid was studied, and it was found that the lipid

peroxide formed by X-ray irradiation *in vitro* had strong biochemical activity[6),7)]. This as lipid peroxide acts as inhibitor of the respiration and glycolysis of Ehrlich ascites tumor cell[8)], as stimulator of the depolymelyzation of DNA, as inhibitor of the growth of bacteria and bacteriophage and as inducer of the transformation of bacteria[9)].

From the results of this experiment, it is clarified that biochemical properties of unsaturated fatty acid are changed by X-ray irradiation. The purpose of this paper is to elucidate the relation between the chemical structure of fatty acid after X-ray irradiation and its biological effect to the membrane system of cells.

## Materials and Methods

Mitochondria were isolated from rat liver by the method of Hogeboom and Schneider[10)] and were washed twice with 0.25 *M* sucrose. The isolated 1 g tissue equivalent mitochondria were suspended just before use in 2 ml of 0.25 *M* sucrose solution as the stock mitochondrial suspension.

Ehrlich ascites tumor cells were harvested 7–9 days after transplantation to strong A mouse and washed 4–5 times with physiological saline solution to remove the contaminated erythrocytes.

HeLa cells are used the strain from Tissue Culture Laboratry, Institute for Infectious Diseases, University of Tokyo.

Fatty acids and their derivatives were obtained from Tokyo Kashei Co. and Sumida Kagaku Co., and were purified. Some of derivatives of fatty acids were also sythesized by routin method in the laboratory of Dr. Y. Shinozaki, Faculty of Agriculture, Okayama University[11),12),13)]. These compounds were served to biological tests. The chemical and

Table 1. Characteristics of fatty acids used in this experiment.

| Fatty acids | A.V. or N.V. | I.V. | M.P. | Preparation |
|---|---|---|---|---|
| oleic acid | 198.2 | 92.7 | — | Tokyo Kasei Co. |
| eleidic acid | 198.0 | 89.2 | 45.0 | Prepared from oleic acid |
| linoleic acid | 200.2 | 176.8 | — | Sumida Kagaku Co. |
| linolenic acid | 201.9 | 261.5 | — | Tokyo Kasei Co. |
| erucic acid | 165.1 | 75.4 | 34.0 | Separated and purified from rape seed oil[11)] |
| brassidic acid | 165.2 | 75.0 | 60.0 | Prepared from erucic caid |
| richinoleic acid | 186.5 | 87.8 | — | Separated and purified from castor seed oil |
| 9, 10-dihydroxyoctadecanoic acid | threo | — | 98.7 | Synthesized[11)] |
| | erythro | | 128.5 | |
| 9,(10)-ketohydroxyoctadecanoic acid | — | — | 74.0 | |
| monoglyceride of linoleic acid | 6.83 | 138.2 | — | Synthesized by Sidhu's method[13)] |

physical properties of these fatty acids and its derivatives are shown in Table 1.

The procedures of X-ray irradiation on fatty acids and its derivatives were as follows. Fatty acids were mixed with 1/10 volume of Tween 80 and emulsified by adding 5 volumes of water. Waring Blender homogenizer was used for the preparation of the colloidal solution (5–10 $\mu$ in diameter of particle). The emulsified fatty acid solutions were poured into a petrie dish (1 cm in depth) and irradiated under condition of maxium back scatter. The physical factors were as follows: 200 kV, 25 mA, filter; 0.5 mm Cu+0.5 mm Al, H.V.L. 1.37 mm Cu, dose rate; 116 r/min., total dose was 3,000 r. After irradiation the fatty acids solution was dried under stream of nitrogen at reduced pressure, dissolved with 20 volumes of purified petroleum ether (b.p. 35–50°C) and placed over night at 5°C. This soluble part (fatty acid) was introduced to brown ampoule with $N_2$ gas. The non-irradiated fatty acid was prepared in the same procedure as X-ray irradiated one except X-ray irradiation. These X-ray irradiated and non-irradiated fatty acids were transfered into petrie dish (11 cm in diameter, 5 mm in depth) at 30°C (relative humidity was 60–85 per cent). After 0, 2, 6 and 8 days these fatty acids were mixed well and served to test their biological effects on the membrane system of cells and to their chemical analysis.

Changes in morphology and in succinic dehydrogenase activity of HeLa cells were observed by the following methods. About 500,000 HeLa cells were inoculated and cultured for 48 hours at 37°C in TD-15 culture tube containing YLE-bovine serum medium (8 : 2), and then the culture medium was exchanged with fresh medium containing 0.1–0.001 per cent of fatty acids. In this case the morphological changes were observed by the inverted phase-contrast microscope. With the similar culture medium, the HeLa cells growing in each tube were incubated with 0.2 ml of sodium succinate(0.2 *M*), 0.2 ml of neotetrazorium solution (0.2 per cent) and 0.2 ml of phosphate buffer solution (0.1 *M*, pH 7.6). After incubation for one hour at 37°C, the activities of succinic dehydrogenase of the cells were measured by the method of Oda[14] and the data were represented by 5 steps according to their activities (optical density of 0.125–0.10, 0.1–0.075, 0.075–0.05, 0.05–0.025 and 0.025—0 at 530 m$\mu$ are indicated by steps of 5, 4, 3, 2 and 1, respectively).

Effect of fatty acids on the cell membrane was observed by the method of Hodes[15] on stainability to nigrosin as described in previous paper. The concentrations of fatty acids were 0.1–0.0025 per cent in final.

The tests of mitochondrial swelling were carried out as described in previous paper[16]: the incubation mixture was composed of 4 ml of 0.15 *M* KCl-0.02 *M* Tris buffer (pH 7.4), 0.5 ml of 0.005–0.0005 per cent and 0.05 ml of stock mitochondrial suspension. The incubation was performed at 25°C for 30 minutes and the change of optical density at 520 m$\mu$ was recorded from 0 to 30 minutes.

Oxidative phosphorylation and physiological swelling-shrinkage of mitochondria were carried out as described in previous papers[16)26]. Simultaneous measurement of the changes at 90° light scattering corresponding to the mitochondrial swelling and shrinkage, and oxygen consumption by rotating platinum electrode were carried out and recorded with a special apparatus constructed by one of the author, K. Utsumi, in the medium consisted

of 0.05 *M* sucrose, 0.02 *M* KCl, 0.02 *M* K-phosphate and 0.1 *mM* of EDTA (pH 7.4) at 25°C. First of all, two ml of the incubation mixture was introduced to the sample chamber of the apparatus and 0.2 ml of stock mitochondrial suspension was added (states 1 and 2). After one minute 0.02 ml of 1 *M* Na-succinate was added (state 4) and one minute later 0.02 ml of 10 *mM* of ADP was added again (state 3). After reverse to state 4 (ADP was phosphorylated to ATP), 0.02 ml of 1–0.1 per cent lipid solutions were added.

The chemical properties of fatty acids used to biological test were determined by the official method of Japan Oil Chemists' Society[17]: in this case acid-and saponification-values were obtained by the use of KOH methanol solution instead of KOH ethanol solution with the 1/10 volume of sample of standard method to analysis. The iodine value was obtained by Wijs method, peroxide value by International Chemical Union method (modified method by Lea). To obtain these iodine-, peroxide-and hydroxyl-values, 1/3 volume of sample of standard method was used to analysis. The content of conjugated double bond was estimated by the ultraviolet absorption. *Cis-Trans* isomerization of double bond was analyzed on infrared absorption spectra, Shimazu Infrared Spectrophotometer, AK-type 275, which had NaCl prism. The sample was measured as KBr discs or Nujol paste.

## Experimental Results

*1. Effects of X-ray irradiated fatty acid on the membrane system and enzymatic activity of cell.*

Table 2. Effects of X-irradiated fatty acid on the succinoxidase activity and blister formation of HeLa cells.

| | Blister formation Final concentration (%) | | | | | succinic dehydrogenase activity Final concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1 | 0.05 | 0.02 | 0.01 | 0.001 | Cont. | 0.1 | 0.05 | 0.02 | 0.01 | 0.001 |
| Oleic Acid 0 | ++ | + | − | − | − | 3 | 0 | 0 | 3 | 4 | 3 |
| Oleic Acid X 0 | ++ | + | − | − | − | 3 | 0 | 0 | 5 | 4 | 3 |
| Linoleic Acid 0 | ++ | + | − | − | − | 3 | 0 | 0 | 5 | 4 | 3 |
| Linoleic Acid X 0 | ++ | ++ | ++ | ++ | − | 3 | 0 | 0 | 0 | 0 | 4 |
| Linoleic Acid 2 days | ++ | − | − | − | − | 3 | 0 | 0 | 5 | 4 | 3 |
| Linoleic Acid X 2 days | ++ | − | − | − | − | 3 | 0 | 0 | 5 | 4 | 4 |
| Linoleic Acid 4 days | ++ | − | − | − | − | 3 | 0 | 0 | 5 | 4 | 3 |
| LinoleicAcid X 4 days | ++ | − | − | − | − | 3 | 0 | 0 | 5 | 4 | 3 |
| Linoleic Acid 8 days | ++ | − | − | − | − | 3 | 0 | 0 | 4 | 3 | 3 |
| Linoleic Acid X 8 days | ++ | − | − | − | − | 3 | 0 | 0 | 4 | 3 | 3 |
| Linolenic Acid 0 | ++ | ++ | + | − | − | 3 | 0 | 0 | 4 | 5 | 4 |
| Linolenic Acid X 0 | ++ | ++ | ++ | + | − | 3 | 0 | 0 | 0 | 2 | 4 |
| Linolenic Acid 2 days | ++ | + | − | − | − | 3 | 0 | 4 | 4 | 3 | 3 |
| Linolenic Acid 2 days | ++ | ++ | ++ | − | − | 3 | 0 | 0 | 4 | 3 | 3 |
| Linolenic Acid 8 days | ++ | + | − | − | − | 3 | 0 | 0 | 3 | 4 | 3 |
| Linolenic Acid X 8 days | ++ | ++ | + | − | − | 3 | 0 | 0 | 3 | 3 | 3 |

HeLa cells show the blister formation with the time after the treatment with linoleic acid in the higher concentration than 0.05 per cent as shown in Table 2. This effect is increased by X-ray irradiation and the blister formation is observed by the treatment of more than 0.001 per cent of X-ray irradiated linoleic acid. In the same series of this experiment the succinic dehydrogenase activity of HeLa cell is accelerated by the treatment of low concentration (0.01%) of linoleic acid and inhibited by higher concentration (0.05%). These effects increased also by the treatment with X-ray irradiated linoleic acid. But the accelerated action of X-ray irradiated linoleic acid is disappeared by the autoxidation of the fatty acid at 30°C in air environment, accompaning with the increase of peroxide value as to be described in later. These effects observed in X-ray irradiated linoleic acid also can be seen in the linolenic acid, but are weaker than that of X-ray irradiated one (Table 2). The membrane-damaging effect of lecithin is very weak, but the fatty acid separated from lecithin has a strong effect. These fatty acids show a strong action to mitochondrial swelling and this action of these fatty acids is accelerated by X-ray irradiation as shown in Fig. 1. In this case the lecithin is emulsified with 2 volumes of water and exposed

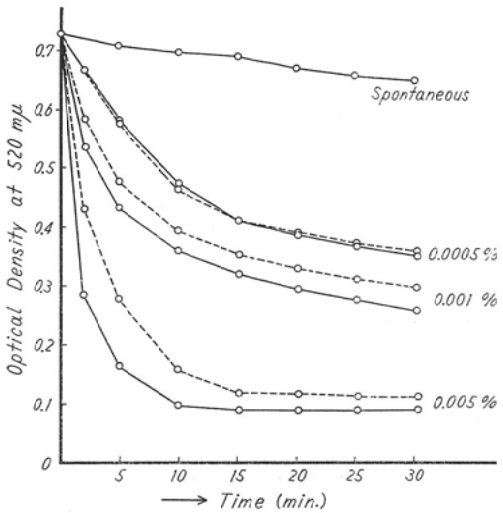


Fig. 1. Effect of fatty acids from X-ray irradiated soylecithin on the swelling of rat liver mitochondria. Incubated at 25°C for 30 min. in the 0.15 *M* KCl-0.02 *M* Tris buffer solution (pH 7.4).
•—• Mixed fatty acids, separated from X-ray irradiated soylecithin; o·····o Mixed fatty acids, separated from soylecithin.

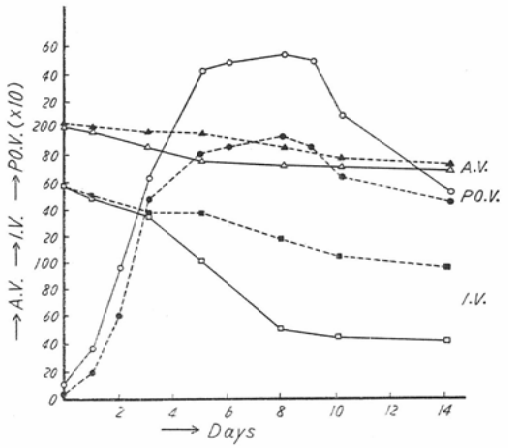


Fig. 2. Changes of chemical properties of X-ray irradiated linoleic acid during autoxidation.

to X-ray as described above on the fatty acid, and the fatty acids are separated from the emulsified lecithin avoiding their oxidation.

*2. Autoxidation of X-ray irradiated fatty acid.*

Linoleic is acid easily autoxidizable acid in air environment and peroxide value is increased after a certain period of lag phase (2 days). In this case the acid and iodine values

are decreased gradually coresponding to the increase of peroxide value. This oxidation of linoleic acid is accelerated by X-ray irradiation as shown in Fig. 2. Stereochemical analysis of linoleic acid is carried out by infrared spectrophotometer (Fig. 3). As shown in Fig. 3, the absorption peaks of X-ray irradiated linoleic acid are observed at 3020–3030, 2860 and 2330 $cm^{-1}$, but these peaks are gradually changed to an indistinct broad curve and the absorption at 3400–3500 $cm^{-1}$ in wave number is increased indicating the increase of OH group (Fig. 3 ; 3, 9 and 14 days). The absorption peak at 910–920 $cm^{-1}$ showing the *cis-trans* form of linoleic acid is observed in X-ray irradiated original sample, but after 3 and 9 days of autoxidation at 30°C the peak changes to a broad spectrum at 970–980 $cm^{-1}$. These results indicate the increases of *trans-trans* isomer and of conjugated double bond with autoxidation. Namely, the autoxidation of linoleic acid at 30°C causes the polymerization of acid due to the formation of lipid peroxide, and the speed of polymerization is increased by X-ray irradiation.

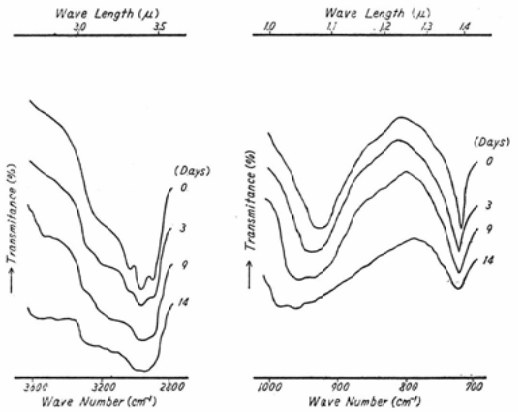


Fig. 3. Changes of infrared spectra of X-ray irradiated linoleic acid during autoxidation.

Changes of stereochemical structure and their speed by X-ray irradiation can be seen on the linolenic, richinoleic and ethyl linoleate. In this case the speed of stereochemical changes of the ethyl linoleate by X-ray irradiation is very small comparing to that of the free fatty acid as described in previous paper[12].

From above-mentioned data it is suggested that the active state of fatty acid to the biological effects is increased in quantity by the X-ray irradiation and that the more biological active fatty acid is the more labile stereochemical structure having a character to form lipid peroxide easily.

*3. Relation between the degree of unsaturation of fatty acid and its biochemical properties.*

The effect of natural glycerides and fatty acid ester having the different number of double bond on the stainability of Ehrlich ascites tumor cells to the dye, nigrosin, is shown in Fig. 4. The stainability of Ehrlich's cell to nigrosin is increased more by the treatment

| | A V | S V | I V | Percent of Stained Cell 10 20 30 (%) |
|---|---|---|---|---|
| Olive Oil | 1.63 | 194.0 | 115.2 | |
| Safflower " | 1.52 | 188.4 | 146.0 | |
| Linseed " | 1.24 | 189.0 | 191.5 | |
| Tung " | 3.32 | 189.7 | 179.5 | |
| Cattlefish " | 1.32 | 182.0 | 195.4 | |
| Ethyloleate | 0.67 | 180.9 | 80.0 | |
| Methyllinoleate | 0.18 | 187.0 | 169.3 | |
| Control | | | | |

Fig. 4. Effect of fatty acid ester and oils on the nigrosin stainability of Ehrlich ascites tumor cells.

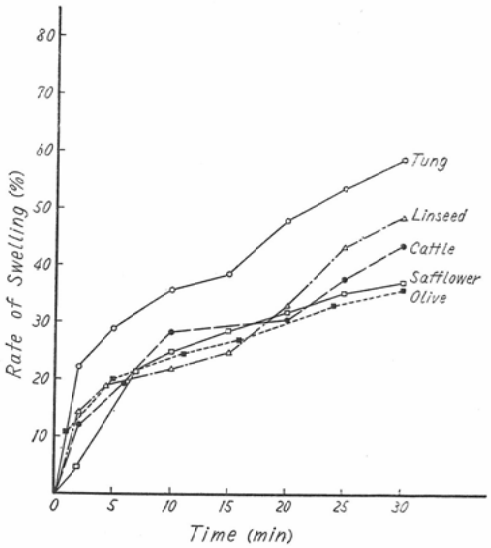


Fig. 5. Effect of various oils on the swelling of rat liver mitochondria. Incubated at 52°C for 30 min. in the 0.15 *M* KC—0.02 *M* Tris buffer solution. Final concentration of oils is 0.1%.

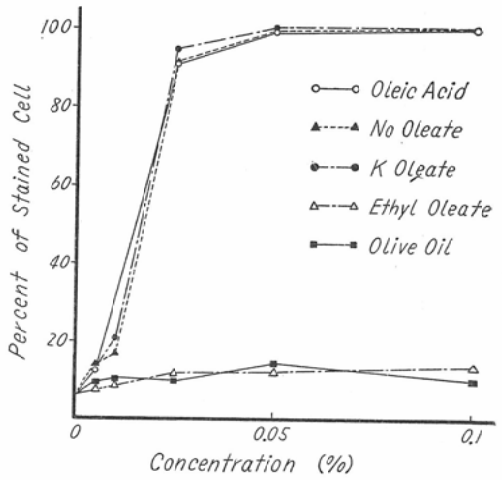


Fig. 6. Effect of various bound types of acyl group of oleic acid on the nigrosin stainability of Ehrlich ascites tumor cells.

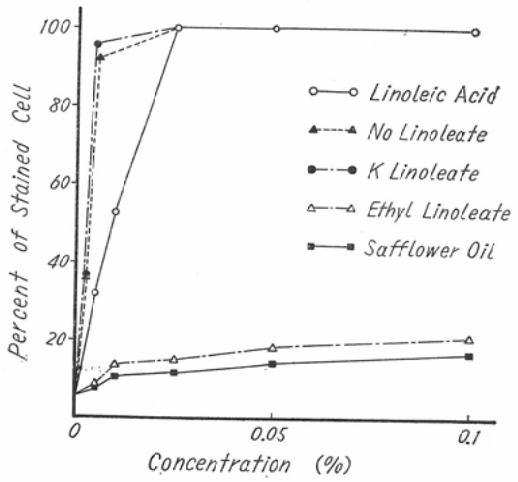


Fig. 7. Effect of various bound types of acyl group of linoleic acid on the nigrosin stainability of Ehrlich ascites tumor cells.

of methyl linoleate than that of ethyl oleate, and is increased by various glycerides in the following order of tung, cattle fish, linseed (drying oil), safflower (semidrying oil) and olive oil (non-drying oil). The effect of various glyceride on the mitochondrial swelling is shown in Fig. 5. The degree of mitochondrial swelling is increased in the following order of tung, linseed, cattle fish, safflower and olive oils. Namely, the effects of the various unsaturated fatty acids to the biological membrane systems are parallel to the degree of these autoxidation.

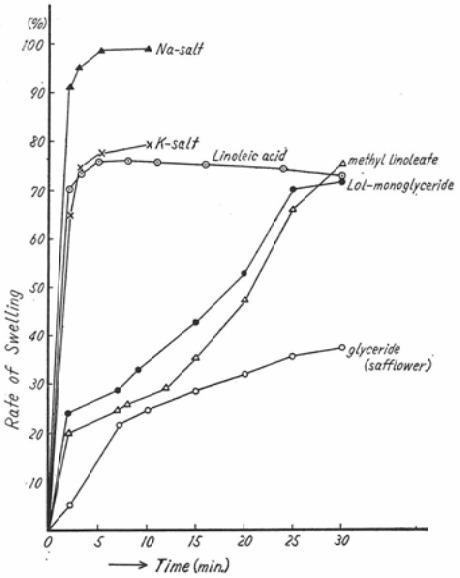


Fig. 8. Effect of various bound type of acyl group of linoleic acid on the swelling of rat liver mitochondria.

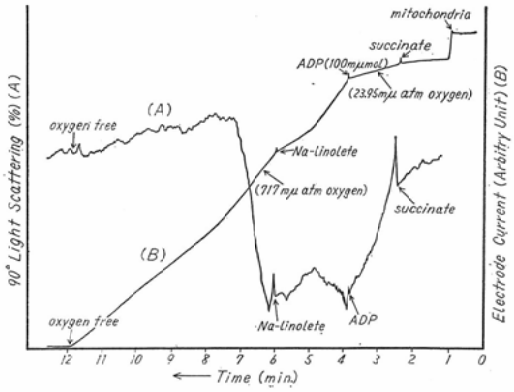


Fig. 9. Effect of various bound types of acyl group of linoleic acid on the respiration and physiological swelling of rat liver mitochondria. Respiratory relese and mitochondrial shrinkage were observed by the addition of the lipids.

Table 3. Effect of various bound types of acyl group of linoleic acid on the uncoupling of oxidative phosphorylation of rat liver mitochondria.

| Lipids | Degree of uncoupling (Ratio of $O_2$ consumption lipid/succinate) |
|---|---|
| linoleic acid | 2.76 |
| Na-linoleate | 2.99 |
| K-linoleate | 3.00 |
| methyl linoleate | 1.67 |
| monglyceride of linoleic acid | 2.01 |
| safflower oil | 1.49 |

*4. State of bound type of acyl group and its biochemical properties.*

Effects of various states of bound types of acyl group on the nigrosin stainability of Ehrlich's cell and on the mitochondrial swelling are shown in Figs. 6,7 and 8. These effects are found to decrease these actions in the order of K- and Na-salts, free and ester forms of oleic and linoleic acids. Fig. 9 and Table 3 show the effects of various states of bound types of linoleic acid on the uncoupling oxidative phosphorylation of mitochondria. The rate of uncoupling is estimated by the ratio of the oxygen consumption after addition of fatty acid derivatives to that of state 4. The rate of uncoupling by various fatty acid derivatives is increased by following order of Na-linoleate, K-Iinoleate free form of linoleic acid and monogluceride of linoleic acid. These experimental results indicate that the biological effect is the stronger in the fatty acid derivatives having the more hydrophilic character

and the lower molecular weight.

*5. Functional groups of fatty acid derivatives and their biochemical properties.*

The degree of mitochondrial swelling induced by the fatty acid derivatives is changed by the functional group and stereo-isomer of the fatty acid. Fig. 10 shows the rate of mitochondrial swelling induced by oleic acid derivatives having the different kinds of functional group. The rate of swelling is more increased by the *cis* or *threo* forms of the acid than that of *trans* or *erythro* forms. The most active form of the fatty acids has a labile stracture, such as 9,10-ketohydroxyoctadecanoic acid as shown in Fig. 10.

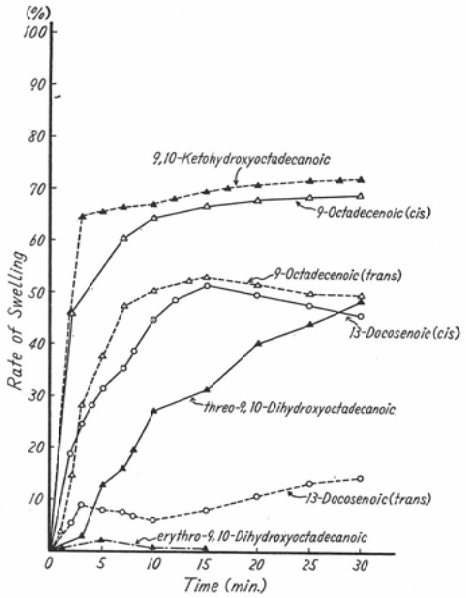


Fig. 10. Swelling rate of rat liver mitochondria by various fatty acids and their derivatives. Incubated at 25°C for 30 min. in the 0.15 *M* KCl-0.02 *M* Tris buffer solution (pH 7.4). Final concentraton of lipids is 0.01%.

## Discussion

It is clarified from the results of this experiment that the X-ray irradiation on the fatty acid in emulsion state accelerates the oxidation of unsaturated fatty acid, and large quantity of the lipid peroxide is rapidly formed after a certain period of lag phase. This X-ray irradiated fatty acid has strong damaging action to the membrane-structure of cell and this action is decreased gradually by the increase of peroxide of the fatty acid by the autoxidation at 30°C. Namely, even the relatively stable unsaturated fatty acid can be changed into the labile and easily oxidizable forms by the action of electro-magnetic wave such as X-ray and γ-ray etc., and the more labile unsaturated fatty acid acts as the more strong membrane-damaging reagent. With respect to the problem of the formation of lipid peroxide with X-ray irradiation, Dugan[18] had studied and obtained similar results to

this experimental results. Lung et al.[19] also studied the effect of γ-ray on the oxidation of unsaturated fatty acid and observed that the degradation of fatty acid was accelerated by γ-ray irradiation. From these experimental results, it can be considered that the shifting of double bond of fatty acid by X-ray irradiation is the initial cause of the formation of peroxide and carbonyl compounds.

The mechanism of the accelerated oxidation of unsaturated fatty acid with X-ray irradiation is not clear, but the process of the oxidation is considered as same as its autoxidation in air environment[20]: the hydroperoxyl group formed with γ-ray irradiation is located in $C_8$, $C_9$, $C_{10}$ and $C_{11}$ of the peroxide isomers of oleic acid, and relative contents of these isomers are the same as that observed in autoxidized oleic acid which has more monohydroperoxyl group in the position of following order of $C_{10}$, $C_{11}$, $C_9$ and $C_8$ isomers. Then the mechanism of the oxidation may be a chain-reaction of free radical produced by X-ray irradiation as was suggested by Polister[21].

The damaging action of X-ray irradiated fatty acid on membrane-systems of cells was most active in the state without peroxide, initial stage of oxidation, and this action was decreased by the formation of lipid peroxide. The toxicity of lipid peroxide was studied by many workers[14),22] and was clarified that the peroxide inhibited the respiration, fermentation[12], mitochondrial oxidase activity[23] and cell growth[6].

The biological effect of the X-ray irradiated fatty acid described in this paper may be due to the toxic action of the lipid peroxide. The peroxide is decomposable compound in the water phase or in cells, but it is considered that the labile compounds penetrate into the cell having a capacity to produce peroxide and act as a toxic substance. The mechanism of the membrane-damaging action of active fatty acid is not clear, but it is considered that the membrane-structure of living cell is constructed by the lipoprotein, and such structure will be denaturated by the surface active agents such as saponin, unsaturated fatty acids and lysophosphatides etc. The molecurar interaction is decreased by the shifting of double bond to methyl end of fatty acid molecule as suggested by Markley[24] and the structural lability of fatty acid is increased by the *cis-cis* stereochemical structure[25]. By these reasons the membrane-damaging action such as accelerated mitochondrial swelling and nigrosin stainability of the cell by the treatment of X-ray irradiated fatty acid would be due to physical properties of fatty acid by free radical or surface activity as shown in this experiment.

## Summary

Changes of chemical structure and biochemical properties of X-ray irradiated fatty acid and relationship beween the chemical structure of fatty acid and its biological action on the membrane-system of cell have been studied and the results are as follows:

1. Unsaturated fatty acid is autoxidazable in air environment, and the increase of lipid peroxide and the decreases of iodine and acid values are observed after a certain period of autoxidation at 30°C. The speed of the autoxidation is accelerated by X-ray irradiation, and the X-ray irradiated fatty acid has a capacity to shift its double bond and to form

the large quantity of lipid peroxide and carbonyl compounds. The more labile fatty acid such as X-ray irradiated one has the strong action to the membrane-system of cell, showing the decrease of succinic dehydrogenase activity and increment of mitochondrial swelling.

2. The stainability of the cells to nigrosin, the degree of mitochondrial swelling and the rate of uncoupling of oxidative phosphorylation are increased by the labile and oxidizable fatty acid derivatives which have double bonds, conjugated double bonds, *cis* form of double bond, and *erythro* form of OH isomer.

3. The membrane-damaging activity of the linoleic and oleic acids derivatives which have different kinds of acyl bond is stronger in the following order of fatty acid: alkaline salts, free fatty acids, monoglyceride, fatty acid ester and triglyceride. In the same bound form of acyl derivatives of fatty acid, the activity is larger in the fatty acid having the more hydrophyric and smaller molecule.

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