CHAPTER 10

# RADIATION

THE STUDY OF THE effects produced by irradiation upon all biological entities has resulted in the accumulation of a large amount of data. Being unsystematized, this information has helped but little to resolve the many physiological and therapeutic problems connected with radiation. New light on these problems can be provided by relating them to the basic concepts we have been discussing.

Other factors also have led us to study the problem of radiation. Its widespread use for the treatment of cancerous lesions with indisputable success in many cases, and the fact that some of radiation's effects appear to be quite similar to those induced by the administration of different lipids, led us to investigate the mechanism through which radiation works and, especially, the possible relationship between radiation and lipids. We will discuss here briefly, some of the results of this investigation.

*Irradiation of Lipids*

We began by trying to determine the effects of radiation upon normal lipids in vitro. As always, we tried to guide the research by theoretical considerations. Investigation of in vivo and in vitro effects of radiation upon proteins in general showed that histones, protamines and alkaline amino acids are most sensitive. These constituents of complex protein molecules have positive electrical character. This relationship between sensitivity to radiation and positive electrical character provided a clue as to where to look in fatty acid molecules for changes induced by radiation. Several positive centers are present in the energetic structure of fatty acid molecules. One is represented by the carbon of the carboxyl. Its positive character is due to its bond to two oxygen atoms. This would explain the exaggerated ionization which takes place at the level of this carboxyl group.

Other positive centers also can be recognized. We have mentioned previously that the positive character of carbon propagates through the chain in an induction effect that causes alternate odd carbons to be positively charged, although the strength of the positive character decreases rapidly with distance from the carboxyl. Since a double bond greatly enhances the energetic character of the carbons linked by it, induction will result in a center in which a more intensive positive carbon is present. Study of the reactions that take place at the double bond in a fatty acid molecule confirms this view, since an electrophilic character predominates

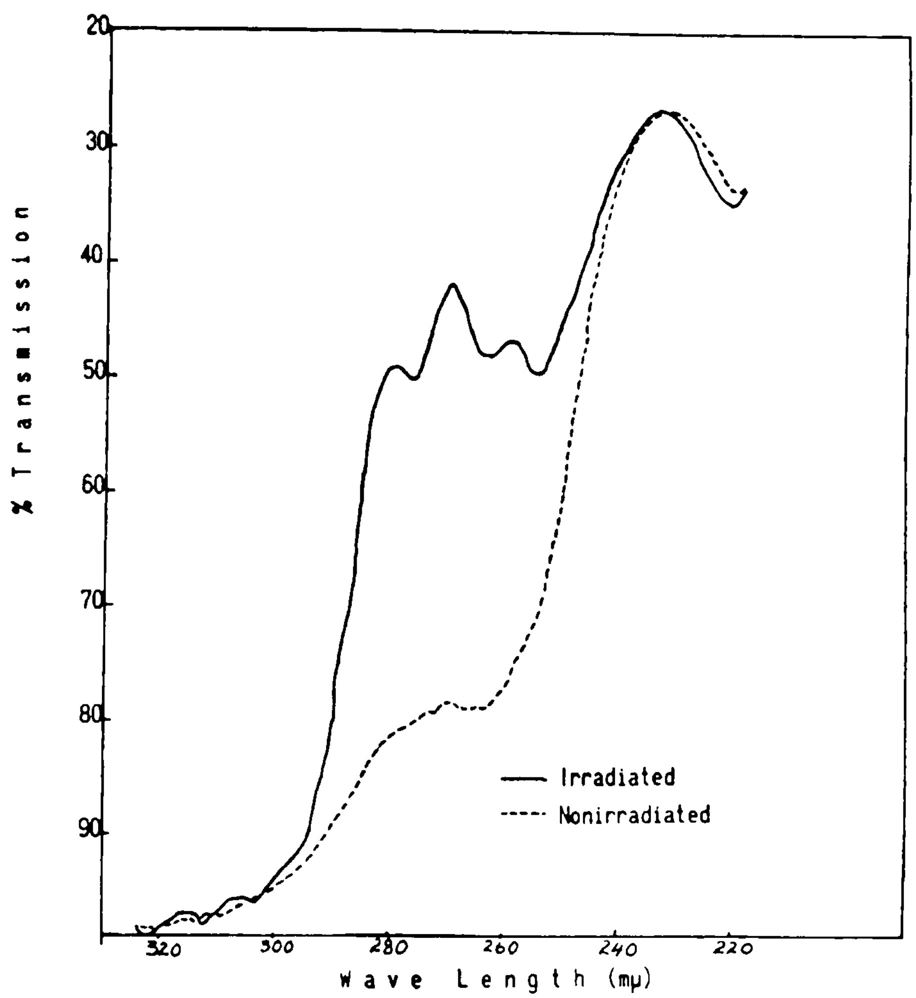


FIG. 79. *Irradiation and conjugation in vitro.* Spectral analyses in ultraviolet of samples of commercially available linoleic acid (with small amounts of linolenic acid present) irradiated with gamma rays from 80 mgr of platinum filtered radium/10 cc, for 6 days at room temperature. For the analyses, dilutions of 0.002% in alcohol, with alcohol as reference, were used. The absorption spectra of the irradiated (——) linoleic acid compared with the nonirradiated (. . . .) shows the appearance of conjugated trienes recognized through the characteristic peaks.

at this point. When treated with sodamine, carbons forming double bonds combine selectively with it, indicating the positive electrostatic character of this formation. Consequently, we thought that the effect of irradiation would be most likely to occur here. This has been confirmed experimentally. We could demonstrate that radiations cause changes especially in the reciprocal position of the double bonds in the molecule.

The results of this research were originally presented before the Fifth International Congress of Radiology in London in July 1951. We will limit ourselves here to a short resume of the procedures and findings:

*Irradiation in Vitro*

a) Radiation of polyunsaturated fatty acids in vitro induces a conjugation of their double bonds, which increases quantitatively with the intensity of the radiation. This has been shown by spectral analysis and by the oxidative fission method. *(Note 1)* Samples of commercially available linoleic acid which, through analysis, have been found to contain variable amounts of linolenic acid, or cottonseed oil were treated with radiations of different sources, such as radium in platinum needles for gamma radiation, in monel metal for beta radiation, thorium X for alpha radiation and X rays. Table XIV shows the results of analysis of the oxalic index. Figs. 79 and 80 show the direct spectral analysis of the samples before and after irradiation as well as the result of their chemical conjugation.

Comparison of direct spectral analyses shows the appearance of an important amount of conjugated trienes in the irradiated samples. Analysis after chemical conjugation of irradiated and control samples shows a greater amount of trienes in the irradiated sample indicating that a process of desaturation also has taken place through irradiation.

A direct relationship was observed between conjugation and amount of radiation. (TABLE XV) The quantity of conjugated isomers, determined by spectral analyses and measured by the oxalic index was seen to increase as radiation was increased either by prolonging exposure time or increasing the amount of radium used.

b) Irradiation of fatty acids appears to induce the appearance of conjugated trienes. When a mixture of polyunsaturated fatty acids, such as those found in cod liver oil, was exposed to a radiation source consisting of platinum filtered radium, the changes were limited to the appearance of conjugated trienes. Conjugated dienes were seen in only some experiments and then only in small amounts. The presence of conjugated members was recognized through their characteristic absorption peaks in ultra-violet anal-

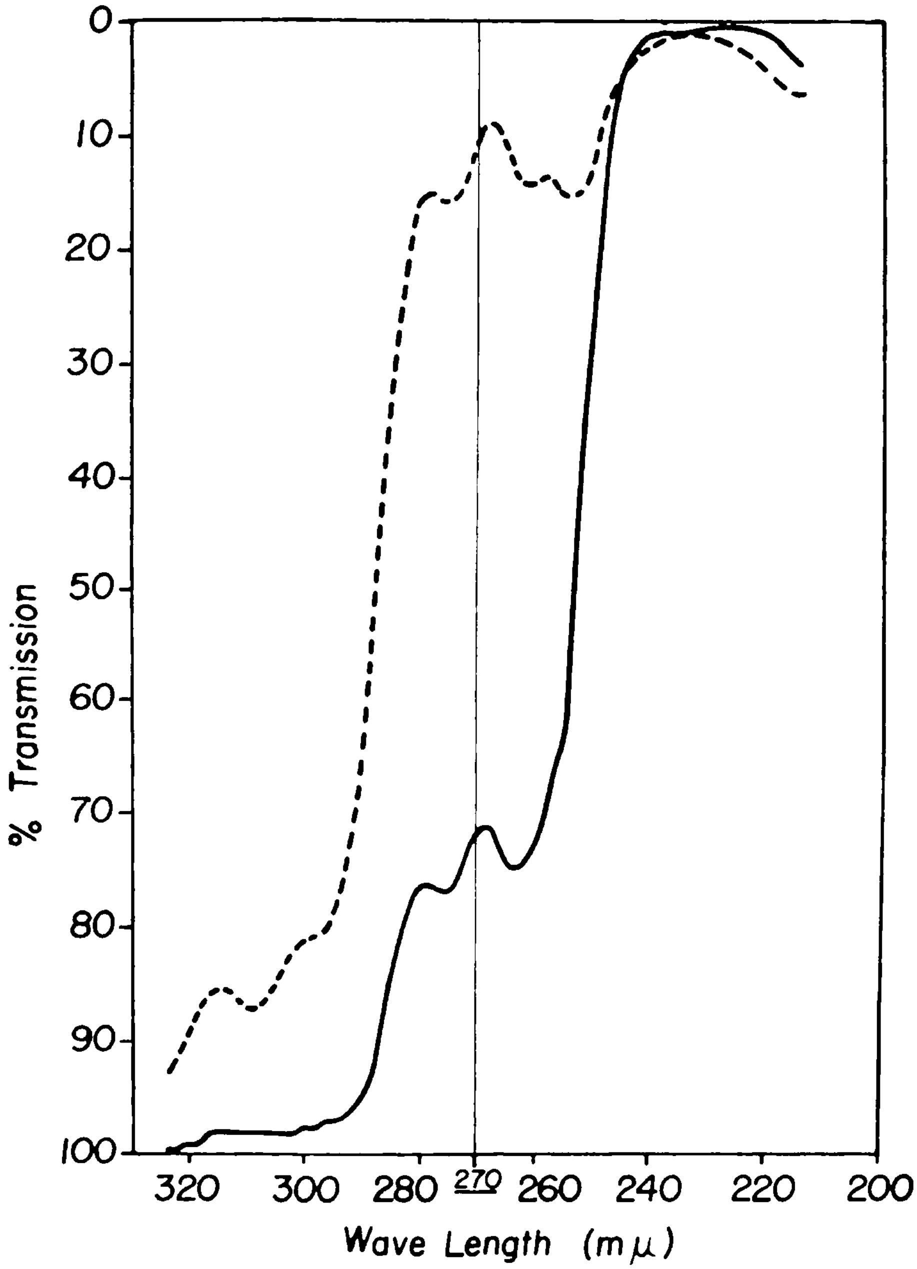


FIG. 80. *Irradiation and desaturation.* Spectral analyses in ultraviolet (0.002% in ethyl alcohol with alcohol as reference) of untreated sample (——) and of the radiated sample (. . . .) both after alkaline isomerization. They show that the irradiation has induced also an increase in the amount of trienes present, which indicates that a desaturation also occurred.

ysis. When the same mixture of fatty acids was treated by the usual chemical methods employed to produce conjugation, *i.e.*, with potassium hydroxide in ethylene glycol or glycerol (41), the spectral analysis showed that the preparation contained fatty acids having between 2 and 6

TABLE XIV

| Fatty Acid | Type of Radiation | Source of Radiation | Exposure Time (Days) | Oxalic Acid mg/g Fatty Acid |
|---|---|---|---|---|
| Linoleic acid | —— | —— | —— | 0 |
| " " | gamma | 50 mg Radium | 6 | 8.3 |
| " " | gamma | 120 mg Radium | 8 | 9.9 |
| " " | gamma | 120 mg Radium | 15 | 13.3 |
| " " | beta | 25 mg Radium in Monel metal | 4 | 9.85 |
| " " | alpha | 150 uc Thorium X | 7 | 4.3 |
| " " | x-ray | 5000r daily—deep therapy machine | 27 | 6.8 |
| " " | x-ray | 5000r daily—deep therapy machine | 53 | 12.4 |
| Cottonseed oil | —— | —— | —— | 0 |
| " " | gamma | 80 mg Radium | 5 | 5.2 |
| " " | gamma | 80 mg Radium | 8 | 8.0 |

double bonds. Figure 81 shows the curves of spectral analysis for such an experiment in which 3 cc. of a cod liver oil fatty acid preparation were treated for six days with 100 milligrams of radium filtered through platinum. In curve "a," of the untreated sample, it can be seen that there is no absorption due to the presence of conjugated members. Curve "b," for the irradiated fraction, shows the typical conjugated trienes, while Fig. 82 shows the result of the chemical conjugation of the nonirradiated preparation with members having from 2 to 6 double bonds.

TABLE XV

EFFECTS OF IRRADIATION UPON THE QUANTITY OF OXALIC ACID PRESENT AFTER OXIDATIVE FISSION

| Fatty Acid | Source of Radiation | Exposure time: Days | Oxalic Acid mg/g Fatty Acids |
|---|---|---|---|
| | 0 | 0 | 0 |
| Linoleic Acid | 120 mg Ra | 4 | 4.5 |
| 10 cc. | " | 6 | 6.2 |
| | " | 8 | 9.9 |
| | " | 15 | 13.3 |
| | " | 20 | 14.4 |
| 10 cc. | 150 mg Ra | 6 | 8.2 |
| | " | 15 | 16.3 |

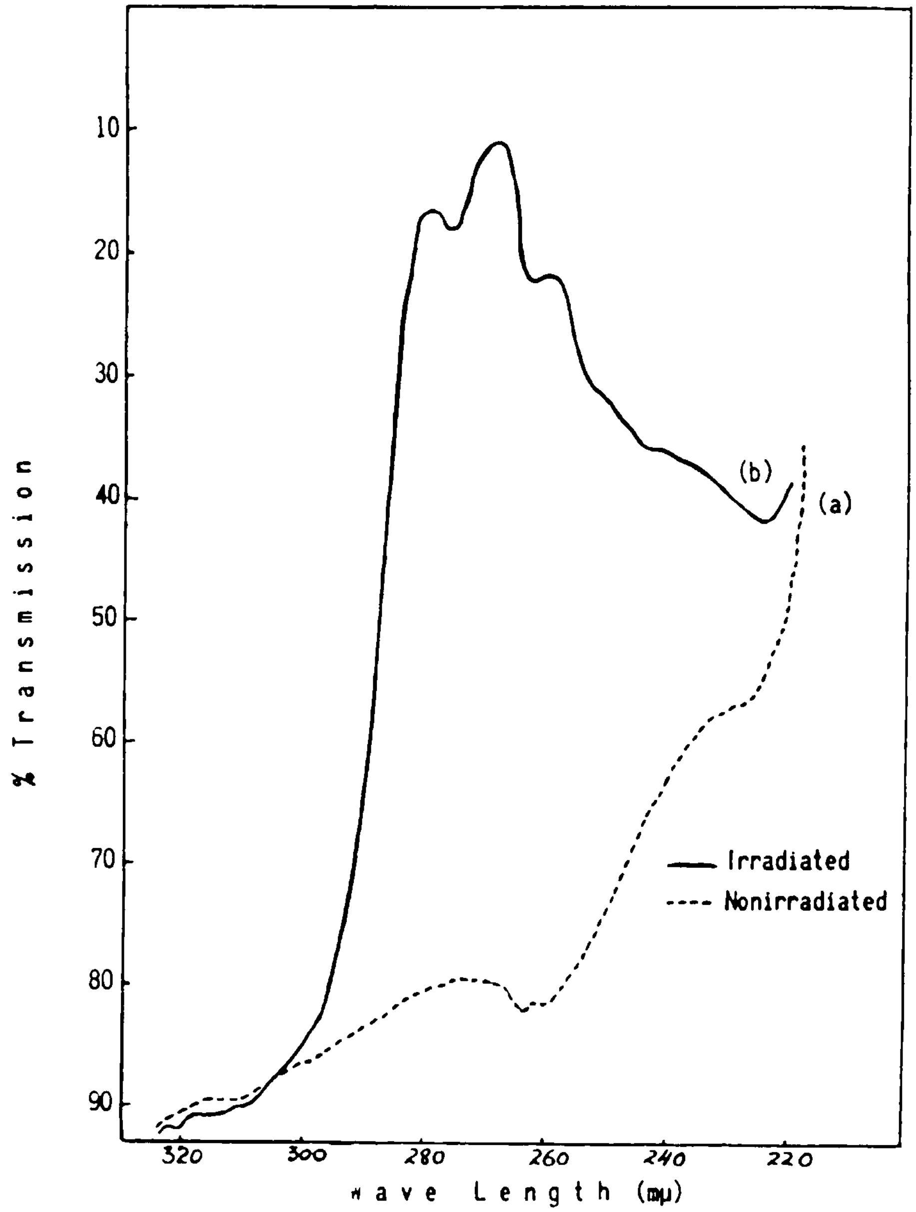


FIG. 81. Spectral analysis (0.002% in alcohol) of a mixture of *fatty acids from cod liver oil,* untreated (. . . .) (a) and irradiated (b) (——) with 100 mg platinum filtered radium/3 cc, at room temperature for 6 days. The analysis shows that the conjugation which takes place leads to the appearance only of conjugated trienes, in spite of the presence of di-, tri-, tetra-, penta- and hexaenic unsaturated fatty acids as shown by the absorption spectrum of the same mixture after chemical conjugation with potassium hydroxide in ethylene glycol. (c) as seen in Fig. 82.

c) The changes induced in fatty acids are essentially the same regardless of radioactive source. Thus the effect upon a linoleic acid preparation containing some linolenic acid was the same with alpha particles of Thorium X, beta rays from radium in monel metal, gamma radiation from platinum filtered radium, and X-rays generated by a 400 kw. therapy unit. Figures 83 and 84 and Table XIV show these results.

*Irradiation in vivo*

d) Irradiation of the body of normal animals killed by decapitation, under ether anaesthesia, produces small amounts of conjugated fatty acids. In early experiments, different organs obtained from slaughterhouses were irradiated and fatty acids examined. In general, even after intensive radia-

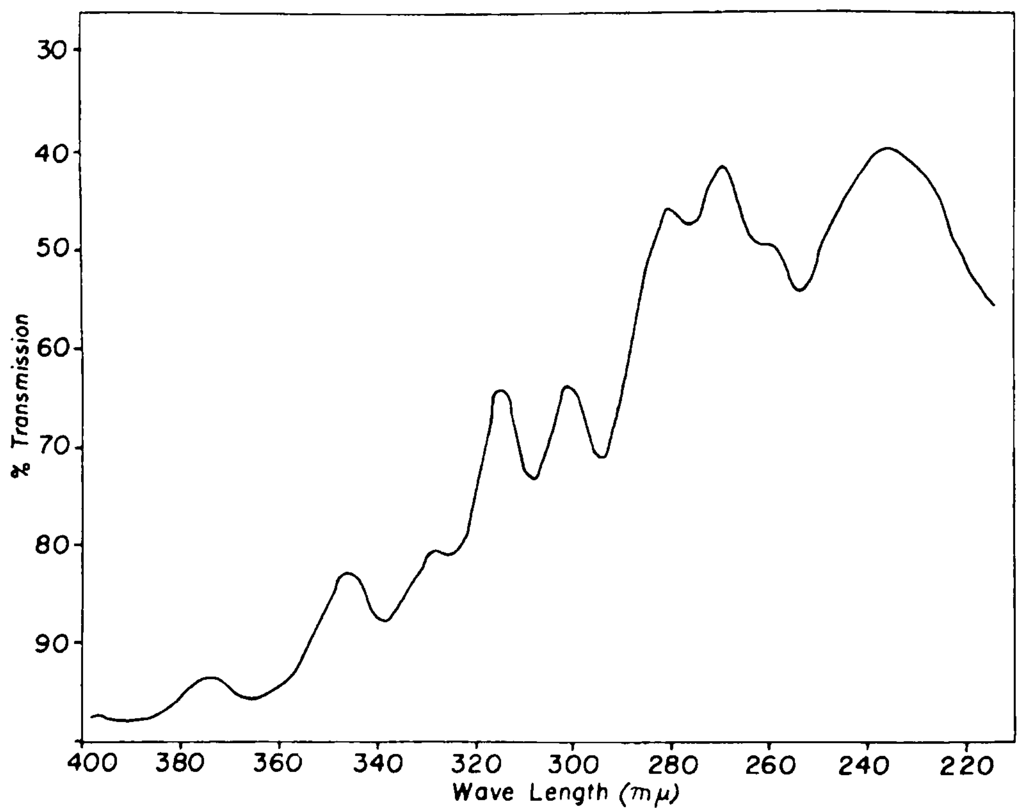


Fig. 82. Curve of spectral analysis of the cod liver oil fatty acids after chemical conjugation, shows the presence of di-, tri-, tetra-, penta-, and hexaenic members.

tion, corresponding to 4,000 r. in one treatment, the oxalic index of fatty acids was never found to be above 1, corresponding to 1 mg. of oxalic acid obtained from one gram of fatty acids.

e) On the other hand, the amount of conjugated fatty acids in the bodies of living animals receiving radiation increases significantly. The following experiment is illustrative. Eighty rats of the same sex, age and weight (about 180 gms.), separated into several groups, were given 1500 r., delivered by a therapy unit with no filter. Four control animals were killed

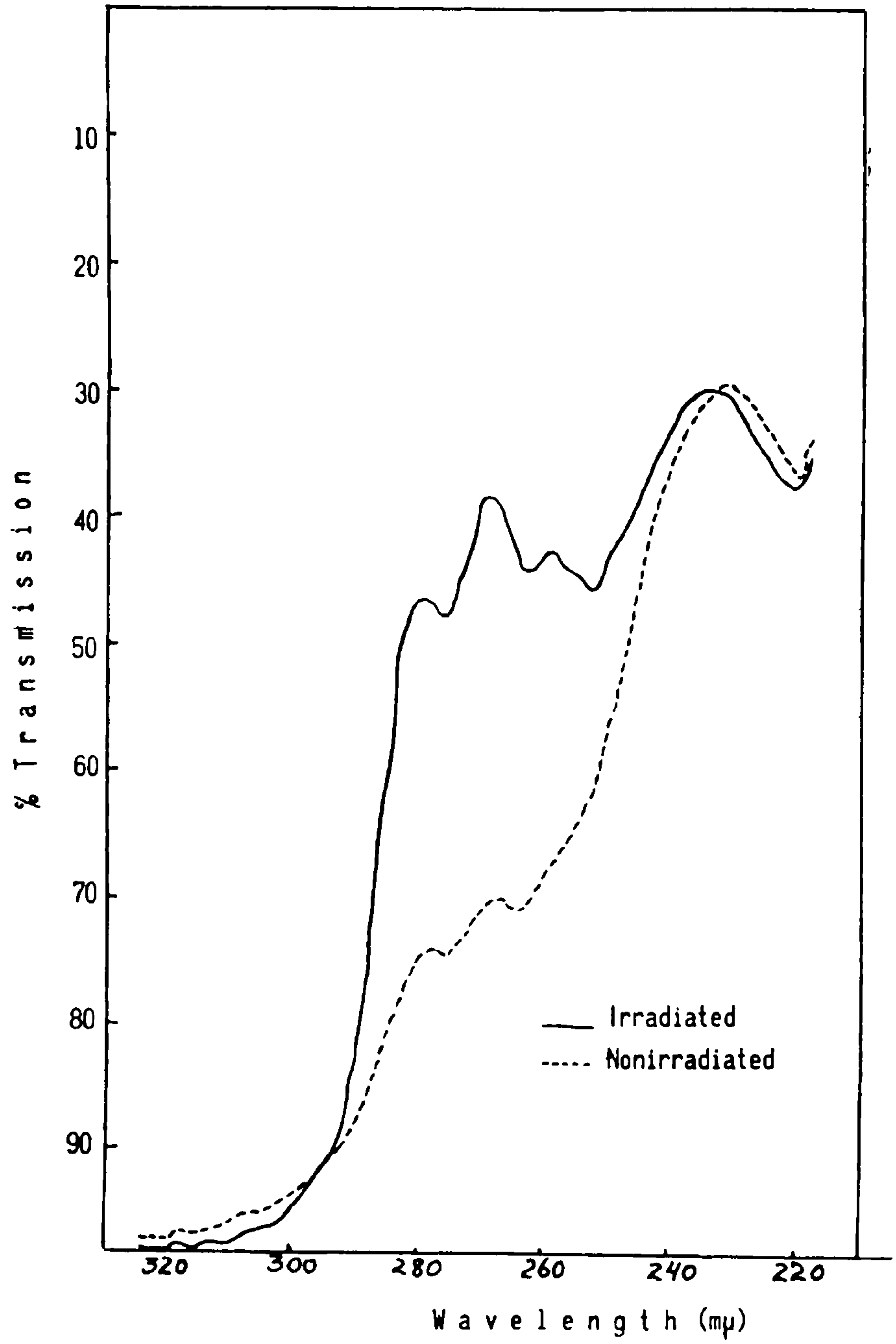


FIG. 83. Absorption spectra (0.002% in ethyl alcohol/ethyl alcohol) in ultraviolet of commercially available linoleic acid (with small amount of linolenic acid) non-irradiated (. . . .) and irradiated (——) with beta particles from 25 mg monel metal filtered radium/10 cc at 37°C for four hours. Some conjugation occurs in the control when kept in the incubator.

before exposure. Groups of four treated animals were sacrificed periodically, starting immediately after irradiation, at 2, 6 and 24 hours after irradiation, and each day thereafter until all animals died or had been killed.

During this time, nontreated control animals, kept under the same conditions, were also sacrificed. The quantity of conjugated fatty acids

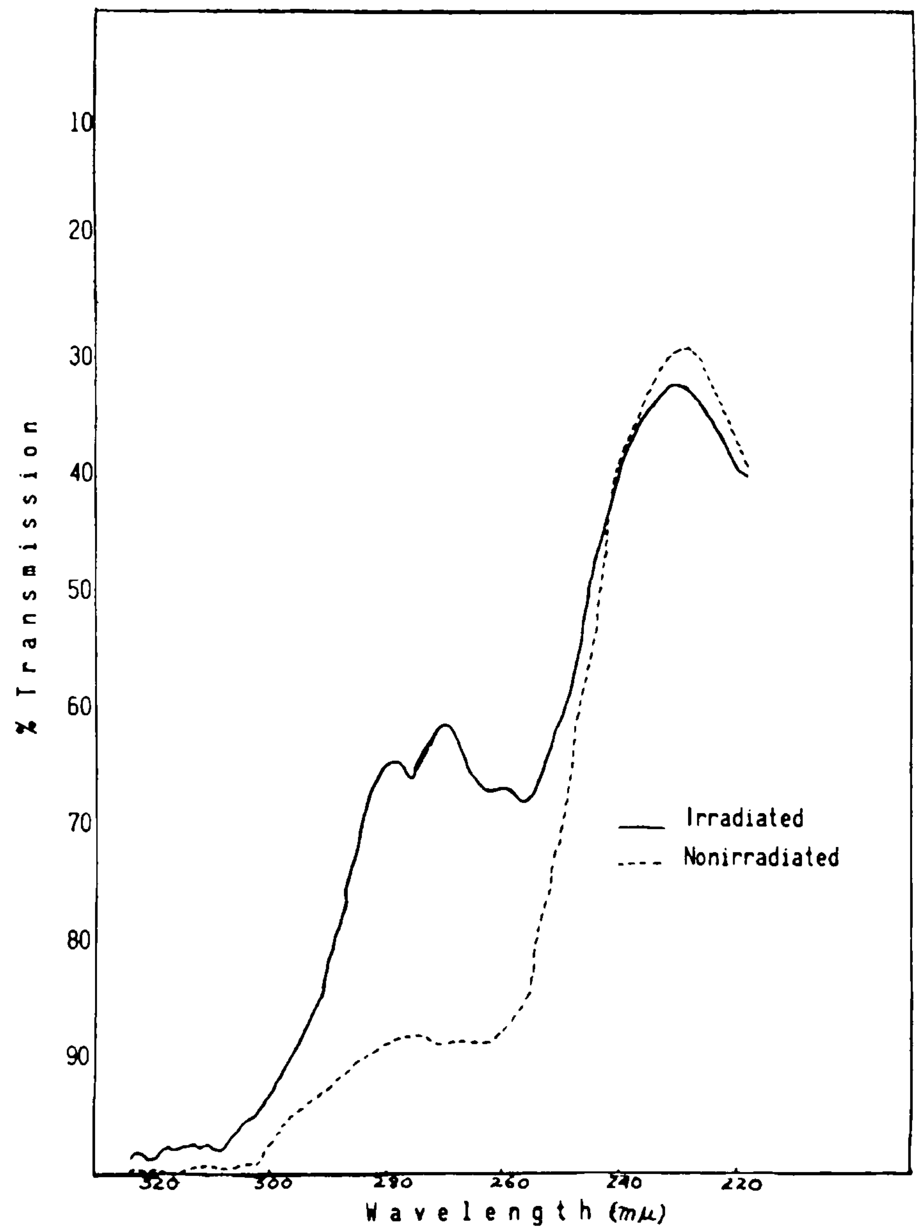


FIG. 84. Absorption spectrum of the same material as in Fig. 83, non-irradiated (. . . .) and irradiated with *alpha particles* from 150 mc Thorium X/10 cc at room temperature for 7 days. (——)

found in the entire body of each individual animal at the time of death was determined by means of the oxalic acid index method.

The oxalic acid index for fatty acids in the bodies of untreated control animals was usually zero. Occasionally there was a variation from 0 but it was always below 0.6. Irradiated animals killed within the first two days showed an irregular increase in conjugated fatty acids, with oxalic acid index values of between 0.6 and 5.1. Three days after irradiation, the oxalic index was above 3 in all the dead or sacrificed animals. The index rose

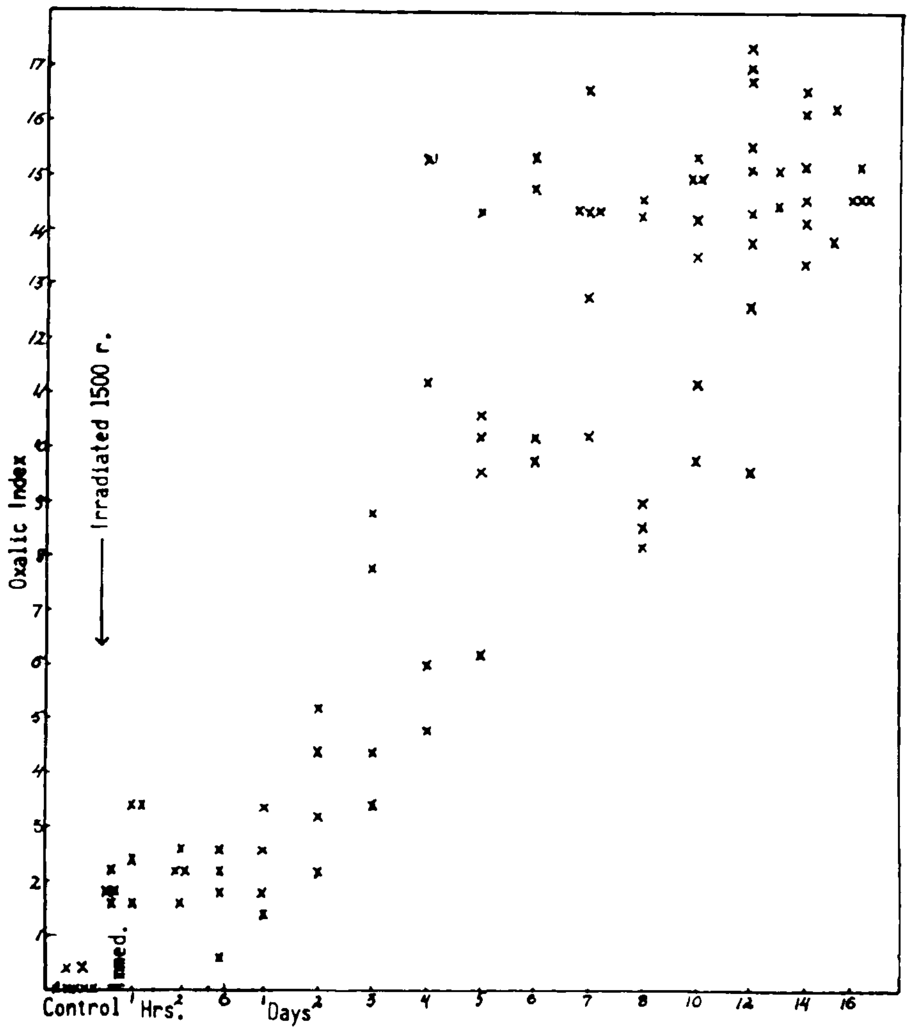


FIG. 85. *Fatty acid conjugation induced by irradiation in vivo.* Changes in the oxalic index of total fatty acids of rats irradiated with a *lethal* dose (1500r). Sacrificed at different intervals, the oxalic index of their fatty acid shows progressively increasing values. The animals die when the index has arrived at a critical value between 14 and 17.

even in animals which showed no visible ill effects at the time they were killed.

The index increased continuously with the passage of time until the animals died. By the fifth day, it was above 6 for all animals and, by the seventh day, with few exceptions, it was around 10. After the twelfth day, it had risen above 12 in most of the animals. In all animals which were

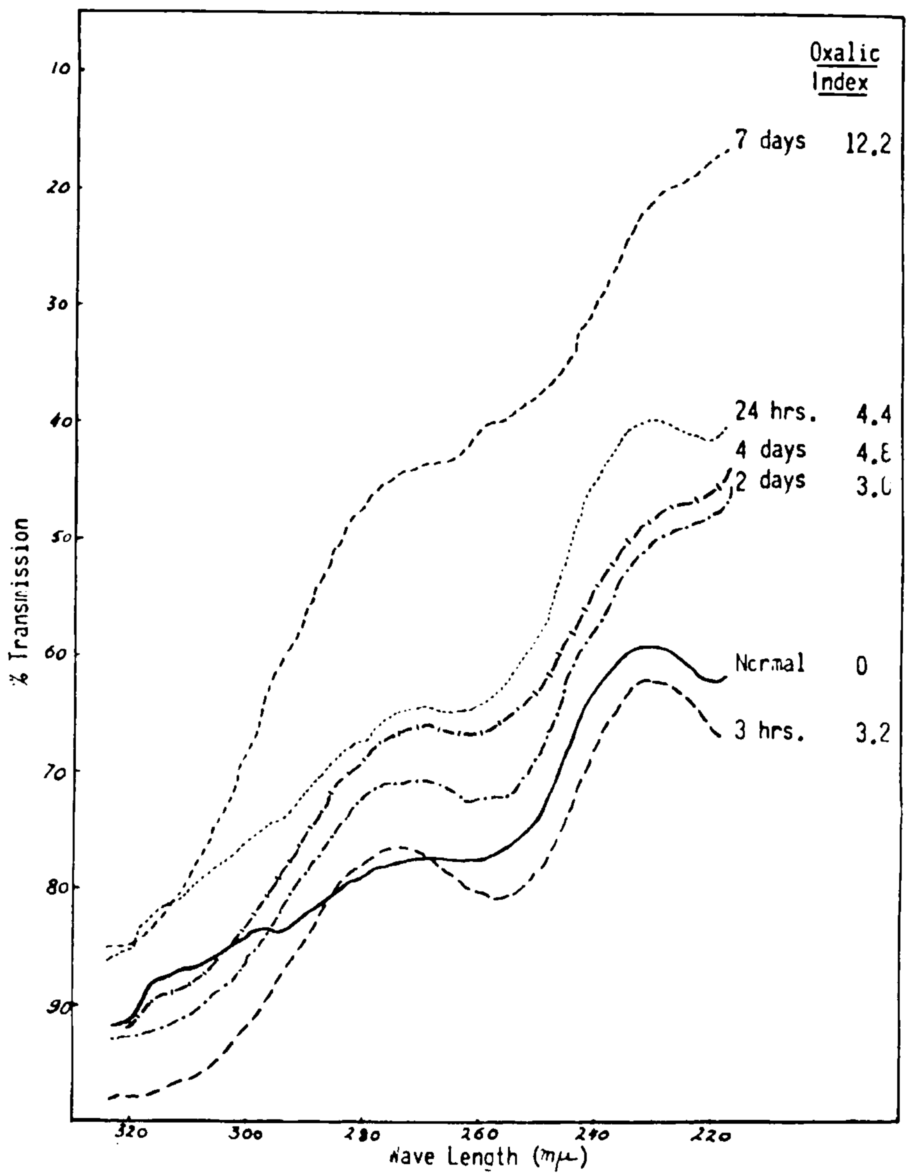


FIG. 86. Spectral analysis (.01 in ethyl alcohol) of the total fatty acids obtained from the body of rats irradiated with 1500r shows changes more manifest around 270 mμ. The oxalic index of the preparation is indicated and shows a parallel increase with the changes in the curve.

sacrificed after the 13th day or which had died at any time, the index showed values between 14 and 17. *(Note 2)* Figure 85 shows these results in the group of rats described in this experiment. These changes were observed when the same procedure was repeated in other groups of animals. These experiments clearly indicated that the quantity of conjugated fatty acids progressively increases in the days following the exposure in animals

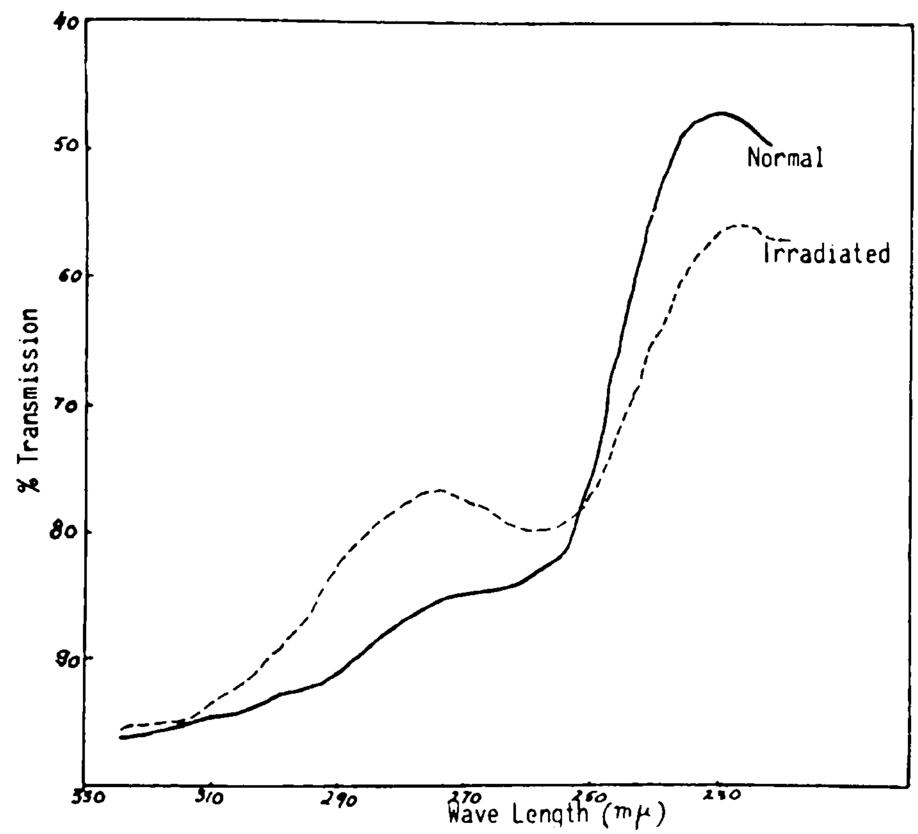


FIG. 87. Spectral analysis (.01 in ethyl alcohol) of the fatty acids of the total body of a mouse irradiated with 1500r, shows an increase in fatty acids with the absorption corresponding to 270 mμ., as compared with the control.

treated with one lethal dose of X-ray. Death occurred when the amount of conjugated fatty acid reached a critical level equivalent to an oxalic acid index value between 14 and 17. The spectral analysis of fatty acids of animals treated with radiation showed changes corresponding to the presence of conjugated isomers. These appear in the samples of fatty acids obtained from the entire body of these animals. *(Figs. 86 and 87)* Still more evident were the conjugated trienes in the fatty acids of organs. Figures 88, 89 and 90 show the difference in such analyses as compared to corresponding untreated controls. The presence of conjugated trienes appears clearly in the characteristic peaks.

The concept of a critical value for the oxalic index is supported by other studies in which the same value is found in animals dying after adrenalectomy or after thermal, chemical or traumatic states of shock. Even in animals dying in superacute shock, within 3 to 5 minutes after being severely scalded in hot water, the level of conjugated fatty acids is higher than in controls.

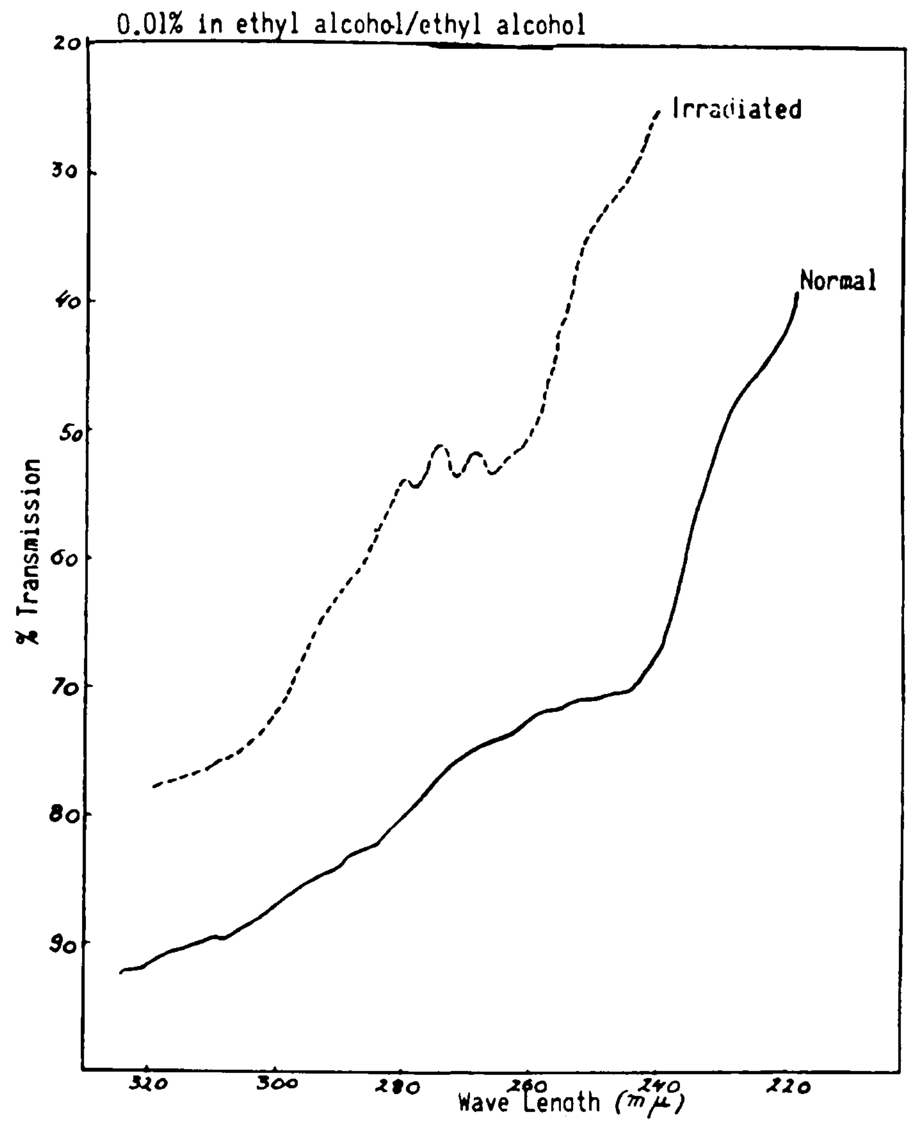


FIG. 88. Spectral analyses (.01 in ethyl alcohol) of the fatty acids of the kidney of a normal rat and of a rat irradiated 6 days previously with 1500r. The peaks characteristic for conjugated trienes are seen.

When the irradiated dose was not a lethal one, that is, below 600 r. in our experiments, the oxalic index increased at first but decreased after about two to three weeks. It never reached the critical value of 14-17. *(Fig. 91, Note 3)*

*Local Effects*

f) We completed the studies of the effects of radiation upon fatty acids in vivo by considering them at the local tissue level. The first requirement

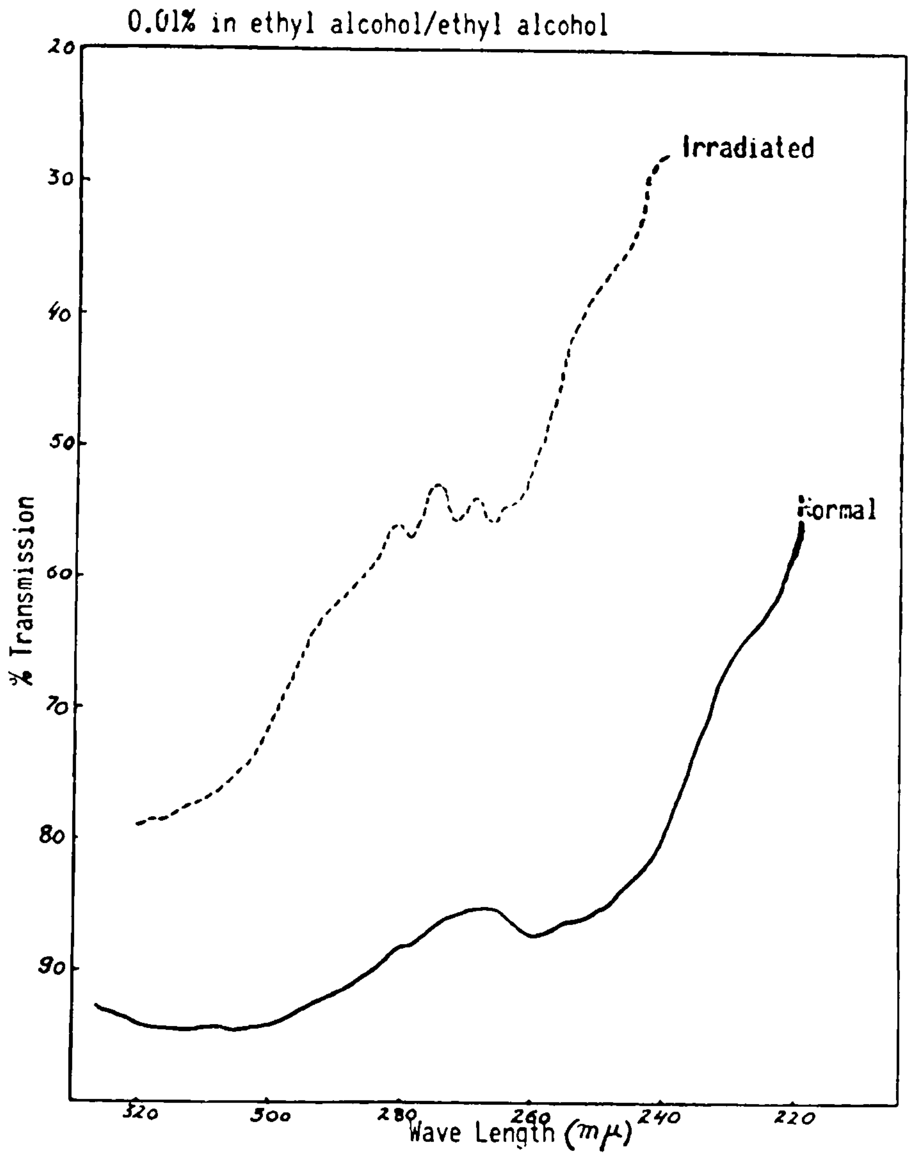


FIG. 89. The spectral analyses (.01 in ethyl alcohol) of the fatty acids of the liver of a normal rat and of a rat irradiated 6 days previously with 1500r shows the appearance of the characteristic peaks of conjugated trienes.

was to establish a radiation procedure which would produce a standardized lesion. When radiations were applied directly to the skin of animals, the individual differences in response were quite marked. These could be explained in part on the basis of age and particularly of sex, the difference

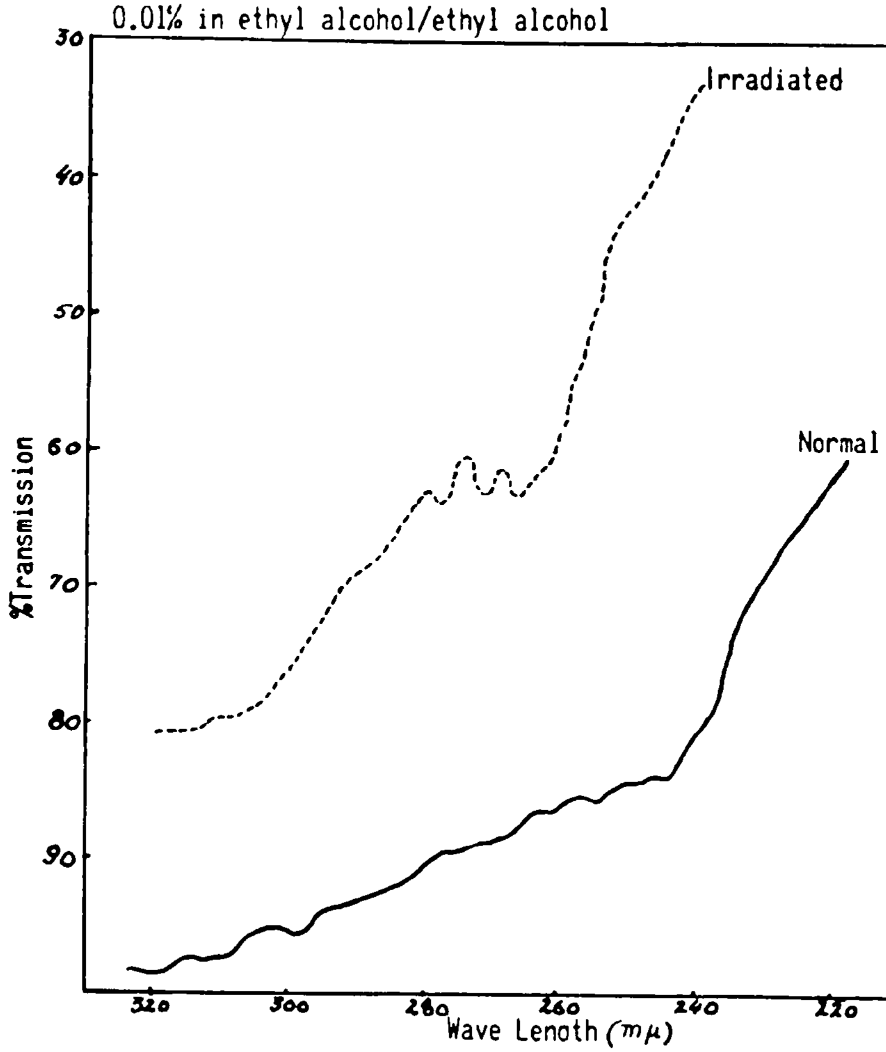


FIG. 90. Shows the spectral analyses (0.1 in ethyl alcohol) of the fatty acids of the lung of a normal rat and of a rat irradiated with 1500r 6 days previously. Peaks corresponding to conjugated trienes are present.

between the skin of male and female rats being manifest. However, there were also pronounced individual differences in animals of the same sex, age and weight living under the same conditions, so that even when the experimental procedure was carefully controlled, the same amount of radiation caused reactions that varied widely from slight erythema to ulceration.

The problem of variability was satisfactorily resolved by radiating abnormal tissues, such as those of a wound, instead of normal tissues. Standardized lesions were first produced and then irradiated. We used the following technique: an area of the skin on the back of male rats weighing around 200 grams was epilated and, under ether anaesthesia, a 2 cm. long incision

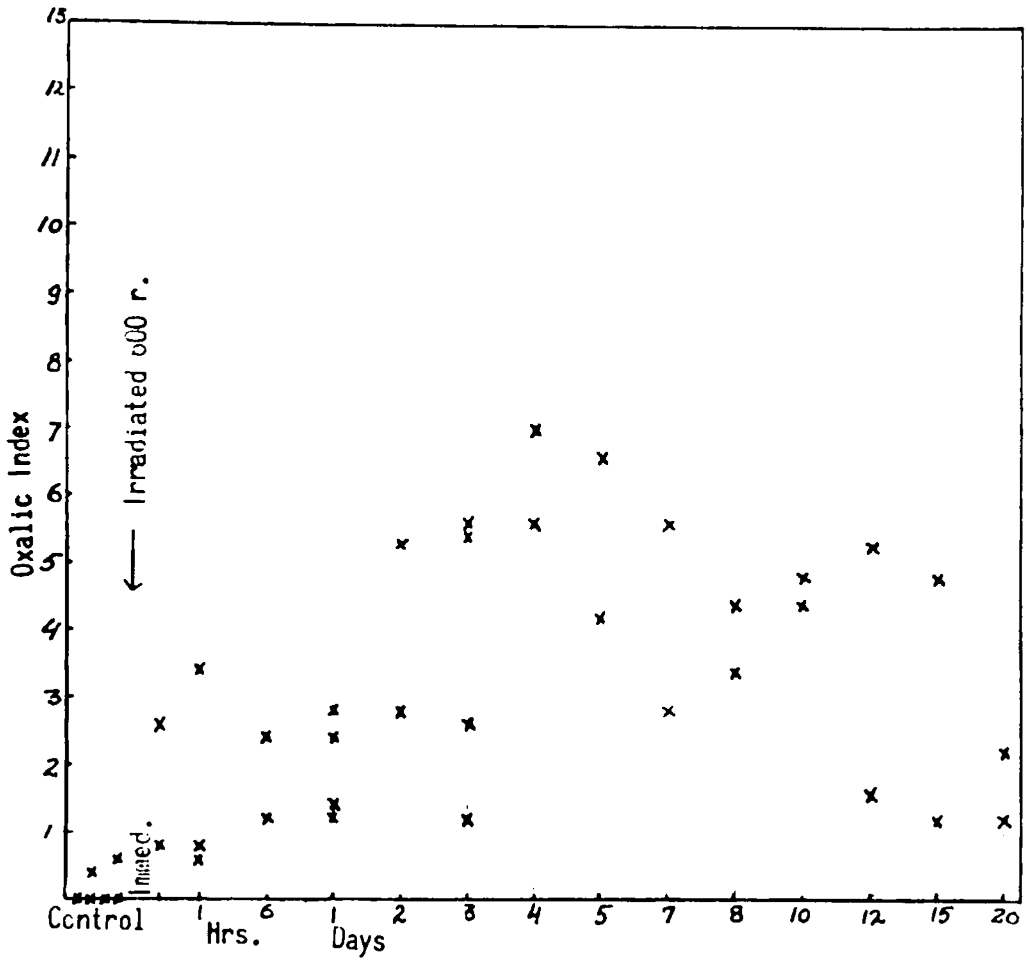


FIG. 91. *Fatty acid conjugation and irradiation in vivo.* The changes in the oxalic index of total fatty acids of rats submitted to sublethal irradiation (600r.). Only a temporary increase in the oxalic index of the fatty acids of the animals is seen, the amounts not reaching the critical values.

was made, penetrating the skin and subcutaneous tissues down to the dorsal aponeurosis. A needle containing radium was then placed between the lips of the cutaneous wound. A thread passed through the hole at one end of the needle was used to fix it to the skin. Two retaining sutures were also used to maintain the radium needle between the lips of the wound. The needle was left in place for the desired length of time and then easily removed with the help of the thread passed through the hole of the needle. The retaining sutures were also removed and the wound left open and undressed.

The length of time that the needle was left in place varied with the amount of radium, the nature of the filtering metal, and the radiation burn desired. We found that 10 mg. of platinum filtered radium had to be left in place for about 90 hours in order to produce a standardized ulceration that would last about four weeks before healing. The same effect was obtained when 25 mg. of monel metal filtered radium was kept between the lips of the wound for only two hours. When monel metal needles were used for only one hour, too great differences appeared between the ulcerations obtained and the time necessary for their healing. A two-hour exposure caused an ulceration which usually required 4 to 5 weeks to heal in control animals. If the needle was left in place for 3 hours, the ulcerations were quite uniform but they required over two months to heal and more than half of the wounds never healed. Failure to heal and more extensive necrosis resulted for periods of exposure beyond 3 hours.

Therefore, we utilized 10 mg. of radium in platinum for 90 hours in one group of experiments, and 25 mg. of radium in monel metal for 2 hours in another group, in order to produce standardized ulcerations that would generally heal spontaneously after 4 to 5 weeks. This technique has been used in several hundred animals for various experiments. The fatty acids of these standardized radiation lesions were studied.

Days after irradiation, the ulcerated lesions were removed along with about one cm. of surrounding tissue and submitted to analyses. It was always necessary to use as many as 5 or 6 lesions to obtain the quantity of fatty acids needed for an oxalic index determination. The lesions were found abnormally rich in conjugated fatty acids. Commonly, indices as high as 40—and in exceptional cases as high as 65—were found (TABLE XVI) in comparison with 0 or 0.3 for normal skin with its subcutaneous tissues.

*Lipids and Radiation Burns*

g) The appearance of conjugated fatty acids as an effect of radiation has posed the problem of the role of these abnormal fatty acids as intermediary agents in the biological changes induced by radiation. In trying to solve it, we compared the effects obtained by administration of conjugated fatty acids with those of radiation at different levels of organization. This study was facilitated by considering the changes which take place in

TABLE XVI

OXALIC INDEX OF FATTY ACIDS OF RADIATION BURNS

| | Elapsed Time | Average |
|---|---|---|
| Normal Skin | | 0.1 |
| Non-treated wound | 24 hours | 2.2 |
| | 48 hours | 3.9 |
| | 72 hours | 2.3 |
| | 6 days | 1.8 |
| Wound with 25 mg. radium in | 2 hours | 1.2 |
| | 24 hours | 6.1 |
| monel metal for 2 hours | 48 hours | 13.9 |
| | 4 days | 19.1 |
| | 1 week | 31.0 |
| | 2 weeks | 46.0 |
| | 3 weeks | 49.4 |

the cellular cytoplasm and nuclei as induced by various substances designated as radiomimetic agents.

It could be seen that apparently all agents which induce radiomimetic effects are lipoids with negative polar groups. The effects of higher polyunsaturated fatty acids, and especially the conjugated isomers, appear to be the same as those of known radiomimetic agents. The similarity between the effects of these fatty acids and those of radiation makes it logical to consider that at least some of the radiation-induced changes result from the intervention of these abnormal fatty acids.

We have seen that the changes induced by fatty acids upon cell metabolism are in large part due to an increase in cell membrane permeability. A similar change of cell membrane permeability could be recognized among the effects of radiation. Following radiation, it could be seen that sodium of the interstitial fluids penetrates into the cells more readily. This was observed when radioactive sodium was used. (42) The cellular vacuolization seen to follow radiation, especially higher doses, represents a corollary of the abnormal penetration of sodium into the cells which partly results from the increase in membrane permeability.

h) At the tissular level, the influence exercised by radiation upon pain was seen to greatly resemble that induced by administration of fatty acids. Radiation efficiently relieves pain that has an acid pattern but it may increase pain of an alkaline pattern. Furthermore, pain which appears following radiation has an alkaline pattern and consequently is increased by further radiation, or administration of unsaturated fatty acids. *(Note 4)*

i) At the tissular level, it could be seen that the area of ulceration of the standard lesions obtained through irradiation of skin wounds was increased by the administration of polyunsaturated fatty acids in general. In some cases the ulceration doubled in size as compared to controls. The administration of fatty acids also markedly delayed wound healing. When the quantity of fatty acids administered was great enough, the wounds did not heal at all. Six daily subcutaneous injections, each of 1 cc. of a 10% oily solution of cod liver oil fatty acids, prevented healing. *(Fig. 92)* The area of ulceration was even greater when only ¼ cc. of a 10% solution of the conjugated fatty acid isomers, obtained through an in-vitro conjugation of the preparation of cod liver oil fatty acids, was administered under the same conditions. This showed that conjugated fatty acid isomers had a more manifest effect upon radiation wounds than the unconjugated acids obtained from the same source.

j) We followed effects of intensive radio and radium therapy in humans at organic levels. In cases with radiation-induced proctitis, mucositis, or epidermitis, the changes observed were seen to correspond to the pattern encountered with fatty acid predominance, and especially to the pattern induced by abnormal fatty acids. The appearance of oxidizing substances in the urine is frequently observed in patients with radiation burns after extensive X-ray therapy. They were almost consistently seen in those cases in which radiation lesions were produced. The administration of fatty acids, and particularly of conjugated fatty acids, to these patients increased the intensity of the lesions.

k) Systemic changes induced by intensive radiotherapy were also analyzed. Here again, the changes followed the pattern observed when there is a predominance of fatty acids, particularly of abnormal fatty acids. The appearance of oxidizing substances in the urine was frequently noted after intensive X-ray therapy and, as mentioned previously, was consistently observed in those cases in which a radiation lesion was produced. Other systemic effects of intensive radiotherapy were seen to include an increase in urinary excretion of surface active substances, an increase of potassium in serum, a retention of chlorides and water, and particularly, an increase in the sulfhydryl index indicating an exaggerated excretion of this group. These changes following intensive radiation are, as previously noted, similar to those seen when a predominant intervention of fatty acids occurs.

Certain of these changes appear to have prognostic importance for radiation therapy. For example, in several cases with very low urinary surface tension, high retention of chlorides and absence of urinary peroxides. The continuation of irradiation led to death. *(Note 4)* This is consistent with the findings in animals that lethal effects of irradiation are directly re-

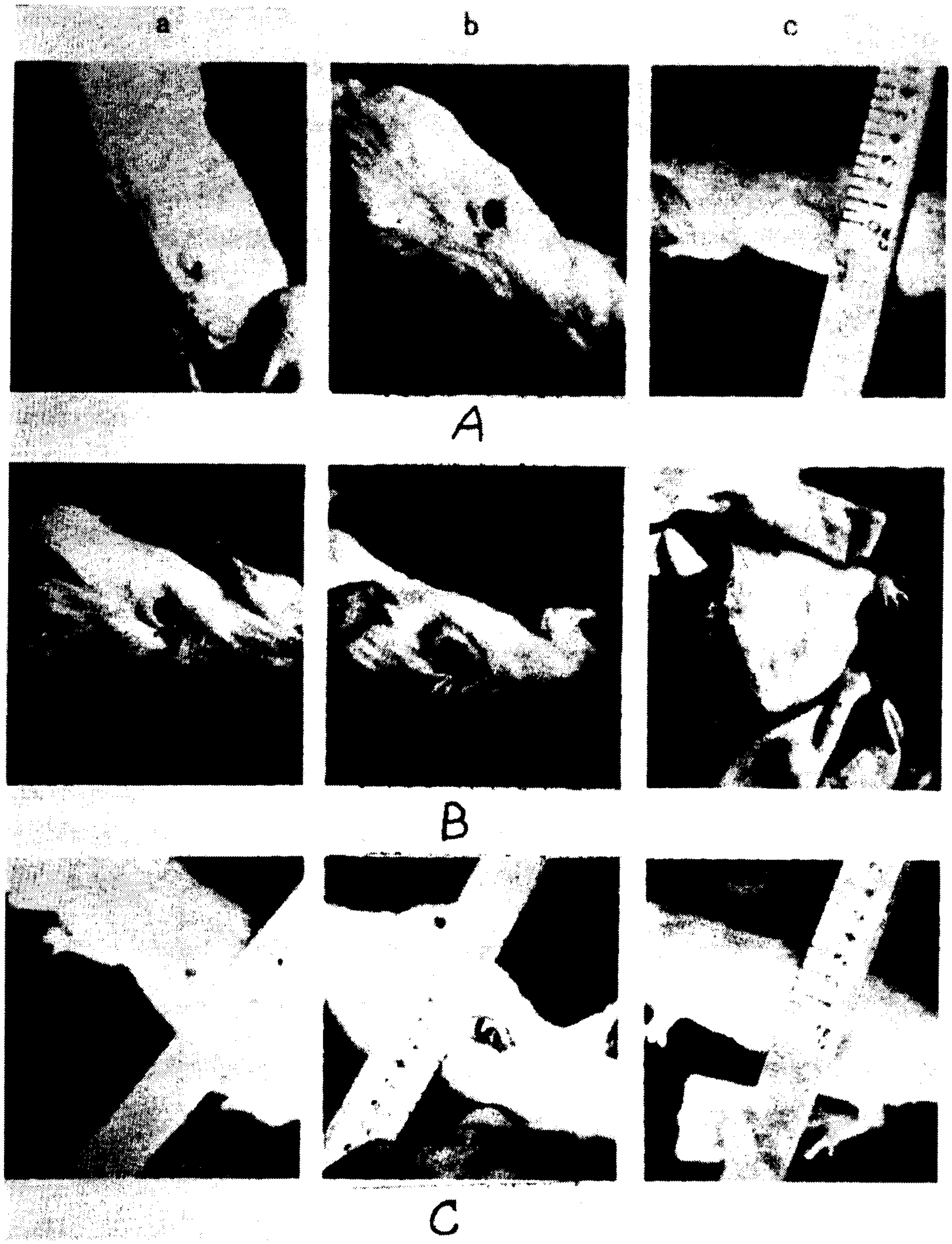


FIG. 92. *Lipids and radiation wounds.* Radiation wounds 5 weeks after exposure to 10 mgr. radium in platinum—for 96 hours. (a) Untreated controls; (b) treated daily with 1 cc of a cod liver oil fatty acids 10% solution; (c) treated daily with 0.5 cc of a 10% solution of unsaponifiable lipid fraction extracted from human placenta. The treatment with fatty acids results in larger lesions than in controls, with no tendency to heal. The treatment with the unsaponifiable fraction leads to a healing of the lesion in around three weeks.

lated to the appearance of large amounts of surface active substances in the urine.

## ROLE OF ANTI-FATTY-ACIDS

The study of the biological effects of radiation also has revealed an important role for anti-fatty-acid agents. The intervention of these substances in the physiopathological processes that occur in the organism under the influence of radiation can be considered to be reactional. They correspond to a response of the organism to changes induced by radiation upon the constituents such as fatty acids.

This antagonism is clearly shown in experiments with animals. The administration of sterol preparations not only reduces the size of ulceration in standard skin radiation lesions but also significantly improves the rate of healing. Insaponifiable fraction preparations from human placenta, beef liver, spleen or blood, as well as from butter, produce such favorable effects. *(Fig. 92)*

If sterols are administered 24 hours after the radium is inserted (or later), the influence upon the dimensions of the ulcer that develops is reduced and is further reduced with increased delay. In some of the animals treated with 1 cc. of a 5% oily solution of the insaponifiable fraction of human placenta in sesame oil for seven days a week, healing with a normal scar was complete within two weeks. Controls, treated with the oil vehicle only, required an average of more than four weeks to heal. Similar effects were obtained with the administration of 1 cc. of a 7% solution of butanol in saline twice a day, beginning with the day of radium application.

The use of small amounts of radiation has, in general, a different effect to that of intensive radiation from this point of view. This can be attributed to the reactive intervention of anti-fatty acids. An exaggerated scar-forming effect, prolonged fibroblastic reaction, exaggerated connective tissue formation, and vascular sclerosis and thrombosis resulting from endothelial proliferation are all part of this long-term response to moderate amounts of radiation. The same effects are produced by anti-fatty acid preparations. All the manifestations are opposite to those obtained with high doses of radiation or fatty acids.

From a clinical point of view, the administration of the insaponifiable fraction preparation had a beneficial radiation effect. Even in lesions that had persisted for years, the pain was observed to disappear after a few days with t.i.d. doses as low as 1 cc. of a 5% solution of the insaponifiable fraction of placenta in oil. In several cases, chronic lesions three to five years old healed in only a few months of treatment.

l) The opposite clinical response to high or low doses was frequently observed in the systemic changes in patients receiving X-ray therapy. While high doses led to a manifest lowering of the surface tension of urine and an increase of the sulfhydryl index, together with the other changes corresponding to offbalance D for small doses of radiation, certain opposite effects related to a predominance of sterols were noted. Of particular interest was the absence of oxidizing substances in the urine, and the changes in urinary surface tension. In all cases treated, a first reaction to radiation was a higher sulfhydryl index and low surface tension corresponding to a fatty acid predominance. When small or moderate amounts of radiation were used, this reaction was very slight and rapidly disappeared in favor of a second change corresponding to a predominance of sterols with high urinary surface tension, for instance. It is interesting to note at this point that this secondary response has been observed especially in those patients for whom radiation also has had a limited therapeutic effect. As we will see, the dualistic interpretation of data furnished by urinary analysis in patients undergoing radiation therapy can be used to guide this therapy.

*Role of Adrenals*

m) The study of the systemic secondary anti-fatty acid response to radiation has led to an evaluation of the intervention of the different anti-fatty acid agents and to the role of the adrenals. It is known that adrenal hormones have a peculiar effect upon the lymphatic system. They induce a shrinkage of the thymus, spleen, and lymph nodes, along with blood lymphopenia. Since similar effects are produced by irradiation, the problem of the part played by an intervention of these adrenal hormones in the radiation response is of interest.

When the adrenals were removed, shrinkage of the thymus and spleen and lymphopenia still occurred after radiation, but was markedly reduced as compared to nonadrenalectomized irradiated controls. Since shielding of the adrenals during irradiation does not alter the effect upon the lymphocytes and lymphatic organs, the role of the adrenals appears to be an indirect one. Adrenal hormonal secretion appears thus to be a response to the systemic changes induced by irradiation. Adrenalectomy would eliminate this secretion and thereby diminish the degree of lymphopenia and the involution of lymphatic organs. However, the secretion does not result directly as an effect of irradiation of the adrenals since shielding does not influence it. Another factor seems to intervene to stimulate adrenal hormonal secretion. The differences between the effects seen in adrenalectomized and nonadrenalectomized irradiated animals corresponds thus to the adrenal response to the systemic changes.

In experiments on rats, we have shown that polyunsaturated, and especially conjugated, fatty acids induce changes in the number of lymphocytes a short time after their administration, and that this is followed by involution of the thymus, spleen and lymph nodes. This seems to occur through the intervention of the adrenals since it takes place to a greatly reduced degree when the same amounts of fatty acids are administered to adrenalectomized animals. The abnormal fatty acids seem to influence the adrenals and their response elicits lymphopenia and involution of lymphatic organs. However, this indirect action through the adrenal glands is only part of the story. Large doses of the same fatty acids will directly induce a certain amount of lymphopenia and involution of the lymphopoetic organs since these changes also occur when these fatty acids are administered in large doses to adrenalectomized animals.

n) All of this research indicates that two of the mechanisms through which radiation acts upon the organism involve changes in lipids. In one, the action is directly through fatty acids; in the other, as a response to these fatty acids, anti-fatty-acid agents intervene. The role of the adrenals appears to be still more interesting considering the nature of the fatty acids produced by radiation. As seen above, the conjugated trienes appear almost specifically as a result of irradiation of mixtures of fatty acids. It was also seen that the corticoids intervene specifically against these conjugated fatty acids. This correlation seems to represent the link between radiation, conjugated fatty acids and the adrenal response. (Ch. 6, Note 17)

*Direct Action of Radiation*

o) In spite of the importance of fatty acids and anti-fatty acids, they represent only one part of the mechanism through which radiation acts. The direct and indirect action of radiation on other constituents also must be considered. The influence exercised upon these constituents can be largely related to various changes. There is a quantitative relationship, for instance, between induction of mutations and the direct impact of radiation on proteins. Changes in fatty acids also are the result of such a direct impact. It appeared interesting to ascertain how much and which of the pathological changes that follow irradiation are due to the direct impact upon lipids and how much to the impact on the other constituents.

The three kinds of biological activity of radiation—through other constituents, through changed fatty acids, and reactional through anti-fatty acids—could be studied at different levels of the organization. We note here a few of the results of these studies.

Below the cellular level, the influence of lipids seems to decrease, causing the direct effect of the radiation on other constituents to appear predominant. For nucleo-proteins and below them, only this last effect seems to occur, the changes induced apparently affecting histones and alkaline amino acids. The close mathematical relationship between the amount of radiation and mutation would seem to indicate that, even at the gene level, only the effect upon constituents has to be considered.

The introduction of anti-fatty acids into the medium in which tetrahymena or suspended cells (as from ascites tumors) were irradiated, served to distinguish the direct effects from those induced through fatty acids. In the presence of anti-fatty acids, vacuolization and even changes in the nuclei seen in the irradiated controls are prevented. The fact that these changes, which characterize the radiomimetic effects, were reduced by anti-fatty acids, indicates the role of fatty acid changes in the pathogenesis of these effects. Among other agents tested, the insaponifiable fraction of organs, and especially of placenta, appeared to be most effective in preventing radiomimetic effects. The high alcohols or glycerol also showed such influence, but to a lesser extent. In complex organisms, the difference of the effect of radiation on fatty acids and on other constituents is increased at the higher levels. At the systemic level, this effect is almost limited to the fatty acids.

The introduction of polyunsaturated fatty acids to the medium greatly increase the toxic effects of radiation, as compared to controls exposed to radiation or fatty acids alone. The proportion of mutations was not changed, however.

The ultimate effect of radiation at different levels depends upon the relationship between three factors: changed fatty acids, other changed constituents, and the intervention of adrenals. The effect of the adrenals is progressively more manifest at the higher levels of the organization. At lower levels, the direct intervention of the abnormal lipids becomes more important than the adrenal response, the latter being less able to act at these levels. At the cellular level, the influence of lipids is still predominant. At the tissular level, the direct lipid effect is still striking, while the influence of the adrenal response is limited to the connective tissue. Although the effect upon the lymphatic constituents (as part of the adrenal response) is important at the organic level, the steroid response becomes more important at the systemic level.

p) On the other hand, it appears possible to vary the amount of the lipidic effect by changing the nature of the radiation. The use of more penetrating rays or of different corpuscles has to be investigated in terms of the relationship between influence upon fatty acids and the effect on other constituents. It could be seen that, in corresponding dosages, the less penetrating radiations had a greater influence upon fatty acids than the more penetrating. The fact explains the reduction of radiation burns directly related to the intervention of fatty acids. Similarly, in a systemic procedure, such as teleradiotherapy, the effect on other constituents is reduced as compared with the direct influence exerted upon the fatty acids.

It is possible that radiations using neutrons would induce an increased direct impact on other constituents without a correspondingly increased effect upon the systemic fatty acids. The skin effect, which is minimal with these radiations, would indicate little intervention of fatty acids.

The unequal part played by lipids at the different levels can be utilized to obtain variations in radiation effects. If effects upon the lowest levels of the hierarchic organization, such as upon histones and basic amino acids, are desired, radiation could very well be the tool to be chosen, because of the small amounts of lipids present at these levels. If the influence could be limited to such action, radiation could be considered ideal for such therapeutic effects. Unfortunately, this is not possible even when very penetrating radiation is used, and the effect of radiation upon lipids still constitutes one of the principal factors which must be considered when radiation is used as a therapeutic weapon.

Thus radiation is not the ideal means for affecting the subchromosomal level, in spite of the fact that it may, through its effect upon proteins, have a profound influence below this level. Its ability to cause a conjugation of fatty acids represents the serious obstacle to its use. In view of this, the effect of radiation upon lipids actually can be considered as an undesirable epiphenomenon whenever the purpose of the therapy is to achieve a local effect at the lower levels. Frequently, the changes which require discontinuation of radiation therapy can be recognized to correspond to abnormal local or systemic metabolism produced by the abnormal fatty acids.

It must, however, be recognized that the appearance of abnormal fatty acids has some advantages even upon protein effects, since indirectly they can make local tissues more sensitive to radiation. We have previously noted that abnormal fatty acids cause changes in the tissue and cellular metabolism which lead to local alkalosis. This local pH change may have favorable results by acting upon the amphoteric proteins and by increasing the positively charged members which apparently are the only ones sensitive to radiation. Indirectly, the intervention of the abnormal fatty acids will thus increase the sensitivity of tissues to radiation.

Before going further, we wanted to emphasize an aspect of the offbalance D for which the study of shock and radiation brought an important contribution. A separation can be made between two phases of offbalance D, one in which oxygen is principally fixed and another in which chlorine is fixed. The first phase, "oxygen," has as characteristic the appearance of peroxides in the urine, and clinically has little noxious manifestation. The other phase, "chlorides," with fixation of this ion leads the serious manifestations as seen, for instance, in shock. For this reason, in radiation the disappearance of urinary peroxides with persisting offbalance D, as seen in the other patterns, will indicate a passage from phase "oxygen" into phase "chlorides," which corresponds to the appearance of a serious condition. (See also Note 4, Chapter 10)

*Radio-Therapy*

The above considerations appear important in the radiotherapy of tumors. The tissular and systemic changes related to the intervention of fatty acids, especially when these changes are sufficiently intense, in themselves can act upon tumors. However, when abnormally intense, they can constitute a serious limitation for continuation of radiation. The manifestations that result from the pathogenic effect of abnormal fatty acids, if intense, can prevent the use of large doses of radiation which would otherwise be necessary to influence a tumor through a direct effect upon the lower levels of the biological organization, histones, nucleo-proteins and even genes. Consequently, the appearance of abnormal fatty acids, which represent an important factor in the biological effect of radiation, can be considered as a favorable effect when we seek to bring about systemic changes and influence pain and metabolism, particularly at higher levels. At the same time, they can also represent a principal obstacle to the more effective use of this same therapeutic agent when one wants to obtain an effect at lower levels.

As for the effects obtained through the influence exerted by fatty acids, they can be decreased by changing the antagonistic relationship between the abnormal lipids and the defense mechanism of the adrenals. With small amounts of radiation separated by long intervals, the intervention of the adrenals, as long as they function normally, can overcome the effect of the fatty acids. With higher doses applied more often, the fate of the irradiated individual depends upon whatever antagonistic factor predominates. With high doses or with a relative adrenal insufficiency, the direct effect of the abnormal fatty acids can become predominant. In that case, the type D offbalance will be more pronounced. It is in such offbalance that subjects die from too intensive radiation. These factors can be of major significance in the intervention against accidental radiation as well as in guiding the therapeutic use of radiation.

Because of the intervention of abnormal fatty acids, systemic radiation does not seem to be the best procedure unless a very intense systemic effect is sought. If this effect is desired, it can be obtained through a method other than radiation. Furthermore, as we have noted above, the conjugating effect of radiation upon fatty acids is almost entirely limited to the production of trienes and dienes. The biological effects of such conjugated fatty acids are more apparent at the tissue level and above it. The energetic value of conjugated trienes and dienes seems to be too meager to permit them to act intensively at levels lower than the cells or nuclei.

In order to have a manifest fatty acid effect, it appears necessary to have an adequate application of radiation. Since an exaggerated systemic action of the abnormal fatty acids may even induce lethal effects, radiation does not appear to be the therapeutic method of choice for an influence exercised through fatty acids. Radiation, however, is more compatible even with a desired localized effect through the limitation of the field in which the changes in fatty acids are induced. In this case, fatty acids may intervene with a lower systemic influence. This accounts for the analgesic action of radiation which probably is related to an effect exercised by local fatty acids. Even here, however, the appearance of an alkaline pattern of pain can lead to undesired changes. In this case, radiation will increase the intensity of pain. This fact reduces the indications for use of radiation even at the tissue level.

*Biologically-Guided Radio-Therapy*

The knowledge of the important roles played by abnormal fatty acids and anti-fatty acids in the biological effects of radiation has suggested a biological guide for radiotherapy. Urinalysis, by reflecting various systemic changes, can serve as a valuable indication of manifestations and processes present in subjects undergoing radiation. The persistence of a pattern related to predominance of fatty acids indicates that the patient has passed into an imbalance that can only be increased by further irradiation and, if it becomes sufficiently intense, may even prove to be lethal, causing the patient to die with symptoms of severe shock. In contrast, a pattern corresponding to the predominance of sterols could be considered as being consistent with a preponderant reactive response, which would indicate that higher amounts of radiation could be used without danger. From a practical point of view, the information given by the urinary surface tension has appeared very valuable. The moment when, and the amount of, irradiation to be given can be determined by these analyses. A low surface tension would contraindicate administration of radiation while high values would indicate that radiation should be increased.

The administration of lipids or lipid-like substances would represent a method of controlling undesirable processes and allowing more effective use of radiation. If the reactive intervention of adrenals appears too strong, a lipoid with negative character could be added to counteract this and, consequently, could increase the desirable effect of the radiation. Subjects receiving fatty acids or sulfur preparations along with radiation have shown intensive local effects with very small doses of radiation. Epidermitis and mucositis were seen in such patients even with doses as low as 600 r. The same intensive effect could be seen in the tumors. The use of lipoids appears indicated when an intensive effect through fatty acids is sought, as in lymphatic tumors. On the other hand, if the effect of fatty acids is higher than can be accepted, and represents a handicap for the desired effect on proteins, then adrenal hormones or other anti-fatty acids must be added. By reducing the effect of abnormal fatty acids, it becomes possible to obtain a more intense impact on proteins and, at the same time, to avoid the otherwise inherent undesired side effects. The choice of the anti-fatty-acid agent must be guided by the level at which the effects of the abnormal fatty acids would make themselves felt. While corticoids act especially upon systemic and organic levels, sterols and other positive lipoids act upon the lower levels. Butanol and similar agents are effective upon local changes, such as pain.

The guidance of radiation therapy, as an example of how this new view may be used to improve therapeutic approaches, will be discussed later.