FIG. 1. Rat liver containing *Trypanosoma equiperdum* inside a blood vessel. × 525.

canaries with *Plasmodium cathemerium* were employed. Small pieces of tissue were fixed in Helly's fluid for 10–12 hr and then washed for 24 hr in running water. The tissues were passed through graded concentrations of ethanol, terminating in absolute ethanol. In order to replace ethanol by butyl alcohol, tissues were passed through graded concentrations of butyl alcohol in ethanol, finally terminating in pure butyl alcohol. By this means, shrinkage of parasites in the tissue is avoided. From pure butyl alcohol the tissues were embedded in paraffin and 5 μ sections were cut.

To demonstrate trypanosomes the slides were stained with equal parts of Kingsley I and II for 5 min and quickly rinsed in two changes of distilled water. The sections were then dipped momentarily in acetone, acetone with eosin, butyl alcohol, followed by three changes of neutralized xylol; clarite was used for mounting. The Kingsley technique of development of a pink color by use of distilled water acidified with acetic acid could not be used because in the preparations described above the coloration of the parasites is totally lost. For this reason, eosin was added to the acetone to potentiate the red cytoplasmic stain of the tissues. Following this procedure the blue-staining trypanosomes were sharply and clearly demarcated.

A section of rat liver with trypanosomes in a large blood vessel is shown in Fig. 1. Because of the length of the organism it is difficult to find parasites in the exact plane necessary to demonstrate photographically what can readily be seen microscopically. The nucleus of the organism stains strongly basophilic, and the cytoplasm appears a lighter blue. Undulating membranes and blepharoplasts are not easily seen.

In staining for malarial plasmodia in sections the same technique is used. After staining, however, the tissues are differentiated for a few seconds in a solution of 1% acetic acid. The slides are then passed through acetone, to which a few drops of eosin solution has been added, followed by butyl alcohol and xylol, after which they are mounted.

The result of this method is shown by a section of bird's breast muscle with numerous nucleated red cells, many of which contain from 1 to 5 trophozoites (Fig. 2).

The Kingsley stain is commercially available as Kingsley I and II or it can be made according to Kingsley's directions. Equal parts of each solution are mixed immediately before use. This stain is excellent for the differential staining of blood and bone marrow films, as well as for blood-borne parasites, such as trypanosomes and malaria. It gives better results in tissue sections of bone marrow than any of the other stains tried by the authors. The Kingsley stain has certain definite advantages over most hematological stains and deserves a much wider use.

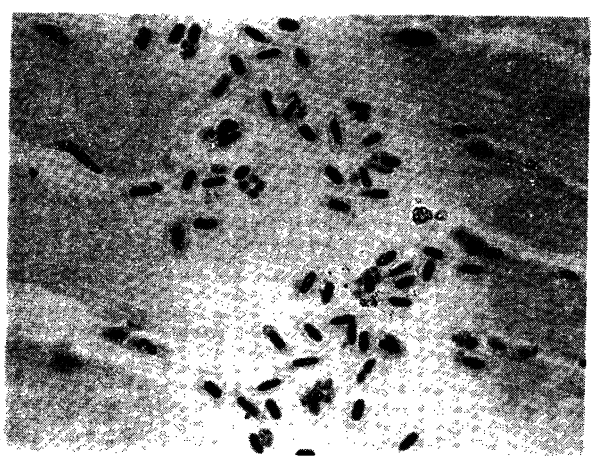

FIG. 2. Canary breast muscle with *Plasmodium cathemerium* inside avian red cells. × 525.

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The Irradiation-Induced Autoxidation of Linoleic Acid[1]

James F. Mead

Atomic Energy Project, School of Medicine, University of California at Los Angeles

In a recent paper, Dubouloz (*1*) stated that peroxides are produced in the skin lipids of animals subjected to x-irradiation and other injury. Consideration of possible mechanisms for this effect and the knowledge that the essential fatty acids, which are universally present in the animal body, autoxidize readily by a free-radical mechanism with the production of peroxides (*2*) have led to the investiga-

[1] This paper is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

TABLE 1

THE EFFECT OF CONCENTRATION OF LINOLEATE ON IONIC YIELD
(Irradiation: 1000 r at 33 r/min)

Concentration of substrate (Molar × 10^3)	Concentration of product (Molar × 10^5)	Ionic yield
10.5	22.9	115
8.50	20.6	103
5.25	13.9	70
3.12	8.7	44
1.30	1.7	8.5
0.78	Not measurable	—

tion of the effect of irradiation on these compounds.

Linoleic acid was selected as the model compound, although theoretically it should be the least affected of the group. Its ready availability, however, made it the compound of choice, with the knowledge that should the study prove interesting further experiments could be made with other more susceptible compounds, such as arachidonic acid.

For irradiation, carefully weighed amounts of linoleic acid were dissolved in 5 ml of alcohol and diluted to 50 or 100 ml with borate buffer of pH 8.5 or 9 (pH had no effect in this range). Irradiation was performed using the Picker 250 KVP Therapy Tube with 20-ml samples of the solutions in slowly revolving spherical Pyrex flasks with indentations for adequate stirring.

Analysis of the solutions was accomplished by comparing the irradiated samples spectrophotometrically with controls that had undergone similar treatment but had not been irradiated. The absorption of light from 300 to 220 mμ by each sample was studied on the Cary recording spectrophotometer. Dilutions were made with appropriate buffer where necessary, and the intensities of the absorption maxima at 231 mμ were recorded and converted by appropriate calculations to concentrations of conjugated dienoic acid[2] and then to ionic yields.[3]

Table 1 shows data on the effect of irradiation at several concentrations on the ionic yield.

Calculation of relationships from the data in the table shows that at constant irradiation rate, concentration of product bears an approximately linear relationship to the concentration of substrate, being greater for the lowest concentrations and falling off somewhat at higher values. The lowest concentration used in this experiment gave no measurable yield (an ionic yield of less than unity would not be detected under the conditions of the experiment). In separate experiments it was found that concentrations below 1×10^{-3} *M* gave no measurable results at 1000 r at 33 r/min.

To determine the effect of irradiation dose on the extent of the reaction, a solution of 5.5×10^{-3} *M* linoleic acid in borate buffer at pH 8.5 was irradiated at constant rate (25 r/min) for different doses. The results are reported in Table 2.

TABLE 2

THE EFFECT OF DOSE ON THE IONIC YIELD

Dose (r)	Concentration of product (Molar × 10^5)	Ionic yield
500	7.4	74
1000	17	85
2000	39	98

The effect of irradiation rate on the rate of the reaction was investigated using a solution of 5.8×10^{-3} *M* linoleic acid in borate buffer, pH 9, which was irradiated in the usual manner with 1000 r at four different rates—540 r/min, 98 r/min, 33 r/min, and 10 r/min. The concentration of product was calculated in each case for the effect produced during the irradiation and also for various times after cessation of irradiation. These data are shown in Table 3. All values are corrected for autoxidation, which is small but significant under these conditions.

TABLE 3

EFFECT OF IRRADIATION RATE ON THE CONCENTRATION OF PRODUCT DURING AND AFTER IRRADIATION

Irradiation rate (r/min for 1000 r)	Concentration of product (Molar × 10^5)		
	Immediately post-irradiation	1 Hr post	2 Hr post
540	2.0	2.4	3.8
98	5.0	6.2	8.4
33	11.8	15.0	18.4
10	28.2	—	—

It is evident that the yield varies markedly with irradiation rate. A similar dependence has been noted by Dainton (*3*) for the radiation-induced polymerization of acrylonitrile.

A complete study of the effect of oxygen pressure on the yield is made difficult by the fact that solutions of linoleic acid in buffer at pH 9 foam very badly. Removal of oxygen was carried out as completely as possible by repeated evacuation (to 25 mm) of the solution, followed by addition of nitrogen. A 5.45×10^{-3} *M* solution of linoleic acid in borate buffer at pH 9 was treated as described above and irradiated at 20 r/min for 1000 r. A control was irradiated under the same conditions but in the pres-

[2] From the E^{mol}_{1cm} of trans: trans 9 : 11-octadecadienoic acid (taken as 3.1×10^4), the concentration of conjugated diene could be calculated if assumed to be of this species. This assumption is supported by the fact that the absorption maximum (231–2 mμ) is the same as that of the above acid, whereas 234 is reported to be the wavelength of maximum absorption for octadecadienoic acids containing cis conjugated double bonds. The assumption that the total yield is given by conjugated diene may give yields about 30% low, since the ratio of conjugated diene formed to oxygen consumed has been shown (*2*) to be about 7 : 10.

[3] The ionic yield was estimated by dividing the number of moles of conjugated dienoic acid formed by the number of "moles" of ion pairs. This number was obtained by dividing the number of ion pairs (about 1.2×10^{15} ion pairs/1000 r/ml for these conditions) by 6×10^{23}.

TABLE 4

THE EFFECT OF CYSTEINE ON THE IONIC YIELD

Concentration of cysteine (Molar × 10^4)	Concentration of product (Molar × 10^5)	Ionic yield
0	8	40
0.8	5.2	26
2	2.9	15

ence of air. The ionic yield for the former was 9, for the latter, 103.

A study of the effect of cysteine on the linoleate system was considered timely in view of the many papers that have appeared recently dealing with the protective effects of sulfhydryl-containing compounds *in vivo* against radiation injury. A further reason for testing this substance was the fact that, unlike more efficient chain terminators such as the hydroquinones, cysteine does not have a high or rapidly changing absorption at 220–240 mμ in the ultraviolet. This fact enables the change in absorption at 231 mμ to be measured more accurately. Three samples of a 1.08×10^{-2} *M* solution of linoleic acid in borate buffer, pH 9, were diluted with equal volumes of buffer, 1.6×10^{-4} *M* cysteine in buffer and 4×10^{-4} *M* cysteine in buffer, respectively. The solutions were irradiated with 1000 r at 25 r/min. Table 4 presents a summary of the results.

It is evident that even the low concentrations of cysteine used in these studies exerted a profound effect on the yield. No attempt was made, however, to determine the exact relationship.

It can be assumed, in all probability, that the mechanism of the reaction of linoleic acid induced by x-irradiation is similar to that proposed by Farmer, Bergstrom, Holman, and co-workers (*2*) for the autoxidation reaction, since this latter reaction has been shown to be initiated by free-radical–producing substances and since the measurable effect is the same in both cases. However, no attempt has been made at the present time to interpret the kinetics of the irradiation-initiated reaction.

It is to be expected that the application of these studies to work *in vivo* will be attended by further complications. Naturally occurring inhibitors may be of great importance, and the measurement of these and other substances possibly destroyed in the reaction will have to be carried out. It is possible that in this case inhibitors will prevent this particular chain reaction from occurring. However, as a model for a type of reaction which may be of importance in irradiation effects, the present investigation may serve as a basis for future studies.

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Comments and Communications

Biological or Psychological Warfare?

AS PRESIDENT of the National Academy of Sciences, I recently received the following cablegram from the president of the Hungarian Academy of Sciences:

We Hungarian scientists have been profoundly shocked to learn of the horrible fact contrary to all human feelings that the United States forces fighting in Korea have used bacteriological weapons to exterminate the peaceful Korean and Chinese peoples. We would most emphatically request you, sir, and all other American scientists in the name of the lofty ideals of science and of moral principles of mankind to raise your voices in protest against this ghastly deed and prevent this crime that is being committed in the name of the people of the United States and with the instruments of science.

ISTAVAN RUSZNYAK
President, Hungarian Academy of Sciences

On Mar. 31 I replied to Dr. Rusznyak as follows:

In your recent cablegram to me you say that the United States forces fighting in Korea have used bacteriological weapons. Many members of the National Academy of Sciences of the United States of America and of its Council are well enough informed to be certain that this assertion is not true. No member of our Academy knows of evidence which supports your claim that the United Nations forces in Korea have waged bacteriological warfare. Therefore, as scientists we cannot accept as facts such unsubstantiated assertions. We regret that you of the Hungarian Academy have not likewise reserved your judgment concerning statements which, in their nature, cannot help but incite passions and hatred, until those statements have been proved or disproved by scientific investigation. We know that the United States has proposed that the International Committee of the Red Cross, a neutral and independent body, make such an investigation on both sides of the battle line in Korea. The ICRC has agreed to do this, provided the belligerents on both sides will cooperate, and has offered to include Asians from nonbelligerent countries in its commission. The Unified Command readily offered to cooperate fully. The Communists so far have rejected the proposal of the ICRC. Accordingly, we urge the Hungarian Academy of Sciences to request the Communist authorities to cooperate in such an impartial and objective investigation by the Red Cross.

Pending such a scientific inquiry we hope you will share our skepticism, as befits men of science. We are saddened by your message, which persuades us that you cannot do so now. This incident strengthens our conviction that only in an open world can men have access to