jected into thyroidectomized rats over a period of several days, have the same order of activity as an equimolar dose of L-thyroxine, as judged from the oxidative metabolism of excised tissues. Triac and Tetrac do not appear to produce an immediate metabolism-stimulating effect, either injected into animals or incubated *in vitro* with tissue slices. At levels which depress oxygen consumption of Ehrlich mouse ascites tumor cells, Triac and Tetrac enhance aerobic and anaerobic glycolyses. However, this effect is not considered specific to the acetate analogs, since thyroxine, triiodothyronine, and a physiologically inactive thyroxine isomer all are capable of producing similar changes.

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Radiation-Induced Alteration of Fibrinogen Clotting Rate and Clot Lability.* (22362)

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Studies of the effect of radiation on the biological activity of proteins have been confined almost exclusively to enzymes. In the case of the latter, an important body of information leading to a better understanding of radiation effects has been obtained(1). Investigations of proteins exposed to ionizing radiation have yielded a wealth of physicochemical data which must, however, be supplemented by examination of the radiation-induced modifications of their biological functions. In the light of the extensive work that has already been done on purified fibrinogen, it was decided to investigate the effects of x-radiation of fibrinogen on its subsequent clotting by thrombin. Fibrinogen has already been studied physicochemically after irradiation in the dry state(2) and in concentrated solution(3). In these studies, the doses of radiation employed were of such high magnitude as to have little practical significance from the point of view of mammalian radiation damage. The present study is concerned with the influence of low doses of radiation.

Materials and methods. Purified bovine fibrinogen (lot No. 55199) was generously supplied by Dr. E. C. Loomis of Parke, Davis and Co., and was shown to contain 90-93% clottable protein. Solutions of this fibrinogen were prepared in 0.15 M NaCl buffered with

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imidazole at pH 7.4 (final buffer concentration 0.015 M). Unless otherwise stated, the fibrinogen concentration in all experiments was 3 mg/ml. Single x-ray doses were given to freshly prepared fibrinogen solutions in Lusteroid tubes. The author wishes to express his gratitude to Messrs. J. L. Weatherwax and L. Stanton of the American Oncologic Hospital for kindly making available to him their x-ray facilities. The conditions of irradiation were 140 kilovolts, 5 milliamperes, 3 mm half-value layer filtration, and 20 cm target distance. The output of the machine was 150 r per minute. The clotting time, *i.e.*, the time required to attain firm-gel endpoints, of irradiated and control solutions of fibrinogen, was determined in triplicate upon the addition of 0.05 ml of saline solution containing, unless otherwise stated, 0.063 unit of bovine thrombin (Topical, Parke, Davis). The clotting reaction took place in 12 x 75 mm Pyrex tubes contained in a water bath at 37.5°C. After allowing the fibrin clots formed in the above manner to remain in the water bath for at least one hour, an equal volume of 10 M urea was added. Each tube was shaken 3 times in order to free the clot from the bottom of the tube. The time for complete dissolution of the clot was then determined. In the experiments with sulfhydryl reagents the latter were added to the fibrinogen solutions prior to irradiation.

Results. Roentgen irradiation increased the clotting time of fibrinogen. The data are given in Fig. 1 and 2 where the logarithm of clotting time is plotted against the radiation dose. In Fig. 1 the dose range is from 0 to 500 r in steps of 100 r; each line represents a separate experiment. In Fig. 2 the dose range is 0 to 100 r in steps of 25 r, and here the 3

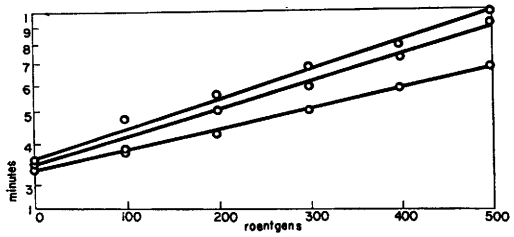

FIG. 1. Clotting time of fibrinogen as a function of radiation dose. Each curve represents a separate experiment.

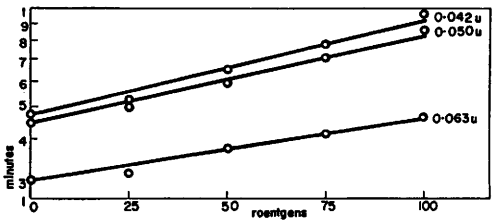

FIG. 2. Clotting time of fibrinogen as a function of radiation dose, using 0.063, 0.050, and 0.042 unit of thrombin, respectively.

lines represent 3 different amounts of thrombin added. In both figures a linear relationship was obtained between the logarithm of the clotting time and the x-ray dosage. Inspection shows that the linear relationship between 0 and 100 r in Fig. 1 is verified by the data in Fig. 2. Fig. 3 indicates that the difference in clotting time between unirradiated and irradiated fibrinogen (500 r) varies inversely with the thrombin concentration. When 0.5 unit of thrombin are added, the difference in clotting time between the unirradiated and the irradiated solution is negligible. Thus, the radiation-induced clotting delay can be prevented by adding an excess of thrombin. The results of irradiation with 500 r on the clotting time of various concentrations of fibrinogen (1 mg/ml to 5 mg/ml) are plotted in Fig. 4. The clotting delay due to radiation was found to be independent of the fibrinogen concentration.

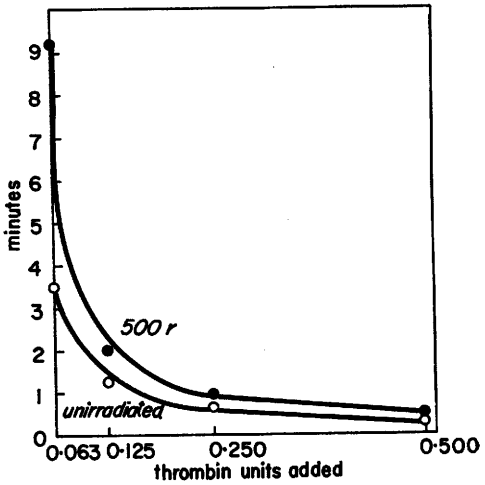

FIG. 3. Clotting time of fibrinogen as a function of thrombin concentration.

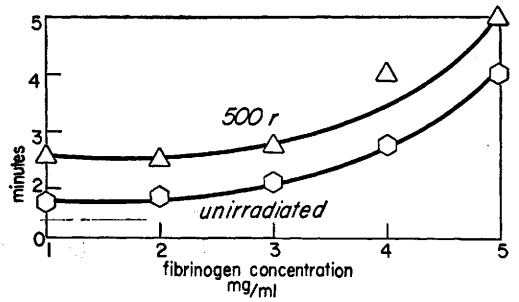

FIG. 4. Clotting time of fibrinogen as a function of fibrinogen concentration.

The extent to which the time of solution in 5 M urea of fibrin clots obtained from irradiated fibrinogen is shortened is indicated by Fig. 5. The plots of time of solution against x-ray dose, at 3 different concentrations of thrombin, reveal that when 0.25 unit of thrombin are added, there is no significant change. When the amount of added thrombin is decreased to 0.125 unit or to 0.063 unit, the time of solution decreases markedly as the x-ray dose increases. Thus, at thrombin concentrations where x-rays prolong the clotting time (Fig. 3), the time of solution of fibrin clots in urea is also decreased.

Addition to fibrinogen, prior to irradiation, of sulfhydryl compounds yielded the results summarized in Table I. 10^{-3} M cysteine or glutathione failed to abolish the radiation-induced clotting delay; they prolonged the clotting time in both the irradiated and unirradiated solutions. Thioglycollic acid (10^{-3} M), as well as higher concentrations of cysteine (10^{-2} M) completely inhibited the clotting process in both the irradiated and control solutions.

Discussion. The radiation-induced clotting delay in fibrinogen solutions may be attributed to a change in the fibrinogen molecule itself. That it is not due to a destruction of the latter will be reported in a subsequent communication, for irradiation failed to alter the amount of clottable protein. Indeed, even with the exposure of fibrinogen solutions to doses up to 130 kiloroentgens, Scherage and Nims (*op. cit.*) found practically no decrease in fibrin yield upon the addition of thrombin. They found that high doses of x-rays altered the fibrinogen to produce a polydisperse material with a higher sedimentation constant and increased viscosity. With the low doses of x-rays employed in the present study no change in viscosity was noted. Consequently, the radiation-induced alterations in the fibrinogen molecule exposed to low doses are of a much smaller magnitude than the effects observed by Scherage and Nims. Nevertheless, the effect of irradiation with low doses sufficed not only to delay the clotting time of fibrinogen, but also to produce structural alterations of the fibrin molecule as revealed by the decrease in time to dissolve fibrin clots in concentrated urea solution.

The progressive increase in clotting time due to x-radiation, leading finally to the establishment of an altered fibrin gel, could mean either that there has been a change in the initial polymerization of the activated fibrinogen, or that a modification, present in the fibrin strands, so changes the further polymerization as to give rise to an altered structural framework of the final clot. Both alternatives are probably at work to produce

TABLE I. Effect of Sulfhydryl Reagents on the Clotting Time of Unirradiated and Irradiated (500 r) Fibrinogen.

Additions	Conc. (M)	Control	Irrad.
None		4′ 30″	7′ 40″
Cysteine	8×10^{-3}	11′ 00″	18′ 10″
	4×10^{-2}	∝	∝
Glutathione (SH)	3×10^{-3}	8′ 15″	12′ 45″
Thioglycollic acid	2×10^{-3}	∝	∝

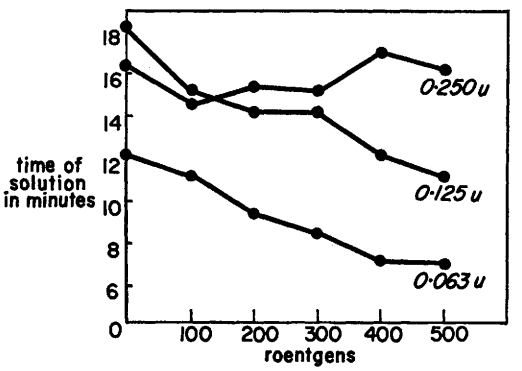

FIG. 5. Time of solution in 5 M urea of fibrin clots obtained from irradiated fibrinogen (500 r) clotted with 0.250, 0.125, and 0.063 unit of thrombin, respectively.

the results obtained. That at least the first possibility is involved can be inferred from the fact that, at high thrombin concentrations, the clotting delay due to radiation is no longer evident. At high thrombin concentrations there is an increase in the rate at which activated fibrinogen appears in solution(4). Apparently, at a high concentration of thrombin, the elaboration of normally activated fibrinogen is sufficiently rapid to overcome the radiation-induced reduction of polymerization. The increase in the difference in clotting time between the irradiated and the control solutions as the thrombin concentration is decreased is then a reflection of the decreased amounts of normally activated fibrinogen formed, the latter being replaced by increasing amounts of radiation-altered material. This modification of the fibrin strands produces an altered clot structure, since these strands comprise the network of a clot which is more highly accessible to the action of urea. At a relatively high thrombin concentration, this effect is also abolished, and this favors the interpretation that the primary modifications produced by radiation become manifest in the early stages of the clotting process.

The effect of low doses of radiation on fibrinogen is apparently not due to an oxidation of sulfhydryl groups, for as pointed out previously, sulfhydryl reagents failed to abolish the radiation-induced clotting delay. Nevertheless, these findings do not exclude the possibility that the polymerization phases of the clotting process are sulfhydryl dependent, since cysteine, glutathione, and thiourea protected radiation-sensitive sites in the fibrinogen molecule at doses ranging from 100 to 600 kiloroentgens(3). The persistence of clotting delay in the presence of similar concentrations of some of the same reagents indicates, however, that sulfhydryl reagents and x-rays act on separate sites in the fibrinogen molecule; both of these sites are essential for clotting to take place.

Summary. Purified bovine fibrinogen was exposed to single x-ray doses in the ranges of 25-100 r and 100-500 r. In each case the logarithm of the thrombin clotting time increased linearly with increasing dosage. The clotting delay due to irradiation was dependent on the thrombin concentration but independent of fibrinogen concentration. Addition, prior to irradiation, of sulfhydryl reagents failed to abolish the radiation-induced clotting defect. Irradiation of fibrinogen increased the rate of solution in concentrated urea of the fibrin clots obtained after addition of thrombin.

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Steroid Secretion by the Isolated Rat Adrenal.* (22363)

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Adrenal vein blood obtained from the rat has been demonstrated to contain several steroid hormones. Corticosterone is present in largest amount but hydrocortisone and aldosterone can also be detected(1,2). Bush found that the ratio of corticosterone to hydrocor-

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