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Effect of Whole Body X-Irradiation of Rats on Net Synthesis of Albumin, Fibrinogen, α₁-Acid Glycoprotein, and α₂-Globulin (Acute Phase Globulin) by the Isolated, Perfused Rat Liver*

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SUMMARY
Adult male Sprague-Dawley rats were exposed to whole body x-irradiation. One hour after 900 r and 2000 r and 2, 4, and 6 days after 900 r, their livers were perfused for 6 hours with defibrinated rabbit blood, with L-lysine-1-14C and 250 mg of glucose infused for the first 4 hours. Net synthesis of several specific plasma proteins was estimated by serological measurements of changes in their concentration in perfusate. Net synthesis of albumin was decreased a maximum of 25% 4 to 6 days after 900 r, which was essentially the same as that evidenced by livers from unirradiated rats fasted for 5 days. Net synthesis of fibrinogen, estimated by chemical as well as serological measurements, was about 1.5 times the normal rate 4 days after 900 r. Net synthesis of α₁-acid glycoprotein was increased at 2, 4, and 6 days after 900 r, with approximately 5 times the normal rate at 4 days. Although α₂ (acute-phase)-globulin was present at low concentration in the plasma of some irradiated rats at the time of hepatectomy, no evidence was obtained for net synthesis of this protein during perfusion of their livers.

Based on the premise that the half-lives of the messenger RNA for rat albumin and fibrinogen are less than 3 hours, our observation that 900 r and 2000 r of whole body x-irradiation failed to suppress synthesis of these proteins is interpreted to mean that x-irradiation in these doses does not damage the DNA of intact rats enough to interfere significantly with transcription of these species of messenger RNA.

Perfusions of livers from irradiated rats did not differ from controls with regard to bile secretion, net changes in urea and α-amino acid nitrogen, oxidation of L-lysine-1-14C to 14CO₂, or incorporation of 14C-lysine into plasma proteins other than albumin and fibrinogen.

Changes in serum proteins following exposure of mammals to whole body x-irradiation have been shown by electrophoretic techniques to consist in general of reduction in albumin and γ-globulin levels and of increased α- and β-globulins (1-8). However, there is little information on changes in concentration of specific plasma proteins following irradiation, and even less is known about whether changes observed are due to changes in synthetic rate, catabolic rate, or both. Thus, decreases in plasma albumin concentration have been associated with increased loss of this protein into the gut because of irradiation-induced intestinal tract damage (7, 9-11), and Zamyatkina (12, 13) has reported a major defect in albumin synthesis by liver slices from rats 4 to 6 days after whole body x-irradiation with 900 r.

Several pieces of evidence indicate that moderate doses of x-irradiation may damage DNA so that normal RNA transcription is impaired. Exposure to 1,000 r of ionizing irradiation has been reported to decrease the priming activity of mammalian DNA for bacterial RNA polymerase (14). In the regenerating liver of rats after doses of 600 to 1,500 r, the following effects of irradiation have been described: (a) reduced rate of synthesis of rapidly labeled nuclear RNA (15-17), (b) interference with the synthesis of specific enzymes (18), and (c) decreased incorporation of α₄-leucine-1-14C into soluble, nuclear, and microsomal proteins (19). These findings, plus the inhibiting effect of 13,500 r of γ-irradiation on genetic transcription in bacteria (20), suggested that whole body x-irradiation of liver donor rats might inhibit mRNA1 synthesis. Since the mRNAs for rat albumin and fibrinogen appear to have half-lives of only a few hours (21), we

1 The abbreviations used are: mRNA, messenger ribonucleic acid; RSA, rat serum albumin.
have sought in isolated liver perfusions to examine effects of 900 and 2,000 r of x-irradiation on the synthesis of these mRNAs by looking for changes in the rates of net synthesis of these proteins following x-irradiation of liver donors. We have also serologically measured net synthesis of \( \alpha_1 \)-acid glycoprotein (22, 23) in the same perfusion experiments.

Effects of x-irradiation on hepatic synthesis of \( \alpha_1 \) (acute phase)-globulin (24–27), on incorporation of \( \text{L-lysine} -1^{14} \text{C} \) into plasma proteins, on the oxidation of \( \text{L-lysine} -1^{14} \text{C} \) to \( ^{14} \text{CO}_2 \), on production of bile, on net urea synthesis, and on net changes in \( \alpha_1 \)-amino acid nitrogen are also described.

**METHODS**

The perfusion system and experimental conditions were similar to those reported previously (21). Liver donors, adult male rats of the Sprague-Dawley strain, weighed between 330 and 400 g; all were allowed drinking water at all times, and in all experiments food was withdrawn 18 hours before irradiation. Animals to be used as liver donors 4 or 6 days after irradiation were allowed food and water ad libitum until 18 hours before the perfusions, at which time food was removed. Fasted controls were allowed no food for 5 days before perfusion.

Perfusion medium was prepared as before from defibrinated rabbit blood (21), so that initial concentrations of rat serum albumin, fibrinogen, and \( \alpha_1 \)-acid glycoprotein would be low enough for accurate serological measurement of net changes during perfusions. Each perfusate contained 2500 units of heparin (heparin sodium U.S.P., Upjohn).

In all perfusions, except those of the 5-day-fasted controls, there was a continuous infusion of 12 ml of Ringer’s solution containing 250 mg of glucose and 15 \( \mu \)C (0.62 mg) of \( \text{L-lysine} -1^{14} \text{C} \) (prepared by standard methods and checked for purity by chromatography followed by autoradiography). In the control experiments with liver donors fasted for 5 days, glucose was omitted from this infusion. As will be discussed below, these controls are deemed adequate for the purposes of this study in that they correspond to extremes of inadequate food intake and possible hypoglycemia which might result from massive whole body irradiation. Infusion, blood sampling, collection of "respiratory" \( ^{14} \text{CO}_2 \), and \( ^{14} \text{C} \) assay procedures were carried out as previously described (21).

RSA and fibrinogen concentrations in perfusate and liver donor plasma were measured with antiserum prepared as previously described (21), but the method of quantitative determination was the single radial immunodiffusion procedure of Mancini, Carbonara, and Heremans (28) as modified by Fahey and McKelvy (29), except that disodium EDTA (Fisher) was added to the buffer to give a final concentration in the agar of 0.2 g/100 ml. The buffer was adjusted to pH 8 with 2 \( \times \) NaOH after addition of EDTA. EDTA was added to correct anomalous diffusion of fibrinogen in agar secondary to formation of small amounts of thrombin, which clots fibrinogen (30). Comparisons of the single radial immunodiffusion method (28) and the quantitative serological precipitin method used in our previous studies (21) showed that both methods gave similar results when used to assay standard solutions of RSA and rat fibrinogen.

Concentrations of \( \alpha_1 \)-acid glycoprotein in plasma from perfusions and from liver donors were also measured serologically (28). The \( \alpha_1 \)-acid glycoprotein was isolated from serum of rats that had received 1.0 ml of turpentine subcutaneously 24 hours before bleeding. The seromucoid fraction containing the \( \alpha_1 \)-acid glyco-
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% CONTROLS (4) 900 r 1 HOURS BEFORE PERF. I21.

O. I400, 6 DAYS BEFORE PERF. (2) 900, I HOUR BEFORE PERF. (2)

HOURS OF PERFUSION

Fig. 1. Cumulative net change in RSA per 300 cm² of body surface area of liver donor rat. In this and in all subsequent figures, numbers in parentheses indicate number of experiments performed, and shaded areas indicate extremes of values for the four control experiments.

Fig. 2. Cumulative disintegrations per min of L-lysine-14C in RSA.

Fig. 3. Cumulative net change in chemical fibrinogen per 300 cm² of body surface area of liver donor rat.

Fig. 4. Cumulative net change in fibrinogen measured serologically per 300 cm² of body surface area of liver donor rat.

Net Biosynthesis of α1-Acid Glycoprotein—Fig. 6 shows net changes in serologically measured α1-acid glycoprotein. No differences from control values were apparent when the liver donor received 900 or 2000 r 1 hour before perfusion. After 900 r there was a notable, large increase in net biosynthesis of this protein, most marked at 4 and 6 days, which is in striking contrast to the small increase seen in perfusions of livers from rats fasted for 5 days.

Absence of Appearance of α1 (Acute Phase)-Globulin in Perfusates—α1-Globulin (acute phase globulin) was not found in the perfusate of any experiment in this series. Our measuring system was capable of detecting this protein at a 1:256 dilution of serum from rats given turpentine subcutaneously 24 hours previously.

Concentrations of RSA, Fibrinogen, α1-Acid Glycoprotein, and α1 (Acute Phase)-Globulin in Plasma of Liver Donor Rats at Time of Hepatectomy—Although blood samples were not available from

tered 1 hour before liver perfusion, neither dose of irradiation altered net increase in RSA from the control values; a 900-r dose 2 days before perfusion was also without effect. Although irradiation with 900 r 4 or 6 days before perfusion resulted in RSA synthesis as low as 75% of that seen in experiments with livers from controls, in no case were values below those obtained with livers from rats fasted for 5 days. Fig. 2 shows that incorporation of L-lysine-14C into plasma albumin was consistent with the net changes presented in Fig. 1, except for a small reduction in incorporation when 900 or 2000 r were administered 1 hour before perfusion.

Net Biosynthesis of Fibrinogen—Figs. 3 and 4 show net changes in chemically and serologically measured plasma fibrinogen, respectively, and Fig. 5 shows incorporation of L-lysine-14C into fibrinogen. The data of these three figures are consistent in indicating increased fibrinogen synthesis by livers perfused 4 days after 900 r of irradiation. Even exposure to 2000 r did not decrease the rate of fibrinogen synthesis below control values.
all rats, Table I shows reduced concentrations of albumin, increased levels of fibrinogen and $\alpha_1$-acid glycoprotein, and small amounts of $\alpha_2$ (acute phase) globulin in plasma from liver donors exposed to 900 r 4 and 6 days before hepatectomy. Except in the case of albumin, there was a good correlation between blood levels of specific proteins in liver donors and net synthesis of those proteins in liver perfusions. For example, 6 days after liver donors were exposed to 900 r, perfusion of the liver from the animal with the higher blood level of $\alpha_1$-acid glycoprotein showed the higher rate of net synthesis of this protein (Fig. 6). On the other hand, 4 and 6 days after liver donors were exposed to 900 r, blood levels of RSA were reduced to 50 to 60% of normal, with only minimal depression of net synthesis of RSA by the isolated livers (Fig. 1).

Incorporation of $L$-Lysine-$1^{14}C$ into Plasma Proteins Other than Albumin and Fibrinogen—After a lag period of 14 hours, control perfusions showed approximately linear incorporation of $^{14}C$ into plasma proteins other than albumin and fibrinogen, reaching $3.2 \times 10^4$ to $3.9 \times 10^4$ dpm by the 6th hour. No experimental results differed significantly from these control data.

Effects of Irradiation on Oxidation of $L$-Lysine-$1^{14}C$ and on Bile Secretion—Cumulative conversion of $L$-lysine-$1^{14}C$ to respiratory $^{14}CO_2$, cumulative bile volumes, and percentage of $L$-lysine-$1^{14}C$ found in total bile were not significantly altered from previously reported (21) control values by x-irradiation. However, in perfusions of livers from rats fasted for 5 days, conversion of lysine-$^{14}C$ to respiratory $^{14}CO_2$ was increased approximately 25%, and bile volume and percentage of the dose of lysine-$^{14}C$ in total bile were each decreased approximately 40%.

Net Changes in Blood Urea Nitrogen and $\alpha$-Amino Acid Nitrogen—As in similar experiments previously reported (21), control perfusions showed essentially linear synthesis of blood urea nitrogen, with production of from 9.7 to 13.2 mg/300 cm² of liver donor body surface area by the 6th hour; experimental perfusions exhibited no significant differences from this pattern. Cumulative net changes in blood $\alpha$-amino acid nitrogen were, in general, similar in control perfusions and in perfusions of livers from irradiated rats. The only significant differences from previously reported control values (21) was an increase of approximately 75% in perfusions of livers from rats fasted for 5 days.

**DISCUSSION**

Much previous work on effects of ionizing irradiation on plasma proteins of mammals (1-8) has lacked specificity, because the experiments involved electrophoretic measurements of relative or absolute changes in serum fractions, each of which contained many proteins. This report presents data on net biosynthesis of several individual plasma proteins, measured serologically, and thereby clarifies the possible role of change in the rate of synthesis in producing the changes in concentration of plasma proteins observed in vivo following whole body irradiation. Our data (Table I), showing reduced albumin concentrations in the serum of liver donor rats 4 and 6 days after 900 r, confirm with a highly
specific serological method the reduction in serum albumin level after whole body ionizing irradiation previously estimated by electrophoretic methods (1–8). However, our studies of net synthesis of RSA by isolated, perfused livers of irradiated rats (Figs. 1 and 2) do not show the large defect in RSA synthesis reported by Zamyatkina (12, 13). In fact, while Zamyatkina found a 60 to 75% reduction from normal 4 to 6 days after 900 r, we found a maximum reduction of only 25% during this period. Perhaps the difference is due to the fact that Zamyatkina's experiments were performed on the less physiological liver slice system.

The fact that our data indicate no lowering of the rate of RSA synthesis below that of rats fasted for 5 days is consistent with the view that hypoalbuminemia in vivo is largely due to postirradiation inanition (4) and to enhanced catabolism or loss into the gastrointestinal tract (7, 9–11), or both. Palmer and Sullivan (7) demonstrated that irradiation of the rat gastrointestinal tract alone decreases the serum albumin level to about the same extent as whole body irradiation and concluded that the effect may have been due either to direct action of irradiation on the intestine or to an indirect effect of some material produced in the gut during irradiation acting at the site of albumin synthesis. An effect of inanition per se seems likely, because serum albumin concentration is known to fall in animals receiving an inadequate diet (39, 40), and malnutrition, as a result of faulty digestion, absorption, or decreased food consumption, is generally found in x-irradiated animals (41).

Precise control of nutritional status in irradiated rats, even by such a technique as paired feeding, is difficult or impossible to achieve. The profound disturbance in gastrointestinal function and nutrition (41) seen after irradiation is hardly to be equated with changes secondary to protracted partial inanition alone. Therefore, we have perfused livers of 5-day-fasted controls without added glucose to represent and serve as a control for the most extreme possible effects of postirradiation inanition and hypoglycemia. It is to be emphasized that under the latter extreme conditions plasma protein synthesis was only minimally altered and to an extent no greater than that seen after irradiation.

Dogs exposed to 300, 600, or 1000 r of whole body x-irradiation have been shown to respond, after several days, with progressive increases in plasma fibrinogen concentration (42, 43). Elevations in the concentration of fibrinogen in the blood of rats exposed to 600 r have been reported by Ponomarev (44). Our data (Table I) indicate similar increases following 900 r, and the perfusion experiments (Figs. 3 to 5) suggest that one major cause of increased fibrinogen concentration after irradiation is more rapid synthesis of fibrinogen.

The rate of net accumulation of serologically measured fibrinogen in control experiments (Fig. 4) is lower than we previously reported (21). We have since found that serial dilution of purified fibrinogen standards (0.3 mg per ml to 0.0125 mg per ml) in a solution of sodium chloride (8.5 g per liter) is associated with progressive severe losses, which were accurately detected and measured with 131I-labeled rat fibrinogen. These losses have been completely eliminated by routinely diluting fibrinogen standards with rabbit serum containing heparin and EDTA at concentrations of 40 units per ml and 2.5 mg per ml, respectively. Previous work with puromycin has shown that release of preformed albumin or fibrinogen from the isolated, perfused rat liver ceased after 2 hours (21). Assuming a low catabolic rate (21), one may consider that net increases in the fibrinogen content of perfusate after 2 hours essentially represent its rate of synthesis by the liver. Based on these assumptions, one may estimate the synthesis rate of this protein to be increased approximately 1.5-fold 4 days after irradiation.

We have found substantial increases in the level of α1-acid glycoprotein in the plasma of rats 2, 4, and 6 days after exposure to 900 r of whole body irradiation (Table I). This appears to be the first study of the response of this specific protein to ionizing irradiation. In view of our results, it now seems likely that the elevations in the α-globulin fraction previously observed in irradiated rats (2, 3, 5) were due, at least in part, to increases in this protein. Perfusion studies (Fig. 6) indicate that elevated plasma concentrations observed in vivo (Table I) are referable in large measure to increased hepatic synthesis. Making assumptions similar to those made above for fibrinogen, one may estimate a 5-fold increase in the rate of synthesis of α1-acid glycoprotein 4 days after 900 r of irradiation. Whereas previous indirect evidence has suggested the liver as the site of synthesis of α1-acid glycoprotein (23, 45, 46), this is the first direct demonstration of net synthesis of this specific protein by the isolated perfused rat liver.

Our observation (Table I) of low levels of α1 (acute phase)-globulin in the blood of rats exposed to 900 r of whole body x-irradiation is similar to the findings of Weimer and Benjamin (25), who reported immunoelectrophoretic evidence of the presence of this protein in the blood of rats after 200 to 800 r of 60Co irradiation. Heim (47) did not find α1 (acute phase)-globulin in starch gel electrophoretograms of sera obtained from rats 3 to 15 days after exposure to 600 r of γ-irradiation, but starch gel electrophoresis is less sensitive than immunoelectrophoresis for the detection of this protein (25). In striking contrast to the marked increase in the rate of synthesis of α1-acid glycoprotein following irradiation (Table I and Fig. 6), α2 (acute phase)-globulin levels in irradiated rats were very low (Table I), and we did not find this protein in perfusates. Since, on the basis of incorporation of isotopically labeled amino acids into immunological precipitates (26, 27), the liver appears to be a site of synthesis of this protein, it appears that synthesis of α2 (acute phase)-globulin either had ceased or was progressing at too low a rate for our measuring system to detect.

Although irradiation caused no change in the rate at which the liver oxidizes L-lysine-14C to 14CO2, there was an increase of approximately 25% after a 5-day fast. This increase may be related to altered pool size of L-lysine accompanying starvation.

As described under "Results," neither irradiation nor fasting altered the rate of urea production by the isolated, perfused liver from previously reported (21) control values. The increased urinary excretion of urea seen in rats after large doses of whole body irradiation (48, 49) appears not to be the result of increased protein catabolism by the liver per se, but is more likely referable to increased release of protein-catabolic products by nonhepatic tissues.

For reasons discussed above, irradiation in the doses we used conceivably could have damaged hepatic DNA enough to interfere with mRNA synthesis. If the half-lives for the specific mRNA for RSA and fibrinogen were 1 1/2 to 4 hours as we have reported (21), and if a significant defect in transcription followed
irradiation, we would anticipate progressively decreasing rates of synthesis of these proteins. Because Figs. 1 through 5 show no such decrease in protein synthesis, we conclude that whole body x-irradiation of normal rats in doses of 900 to 2000 r does not damage hepatic DNA in a way that significantly interferes with its template function for the specific mRNA for RSA and for fibrinogen. Since our preliminary (unpublished) data indicate that the half-life of the mRNA for α₂ acid glycoprotein is less than 2 hours, this conclusion is also applicable to the mRNA for this protein.

In view of our findings, it is important to note that Harrington (14) reported that mammalian DNA, irradiated in whole cells or in vitro, exhibited significant decreases in priming activity for a bacterial DNA polymerase; however, she noted a decrease in priming activity for a bacterial RNA polymerase only after irradiation of the DNA in vitro, and even the effect she observed on the activity of irradiated DNA for DNA polymerase was not demonstrable if mammalian instead of bacterial DNA polymerase was used (50). Further evidence for the failure of ionizing irradiation administered in vivo (in the approximate dose range we have used) to inhibit RNA transcription from DNA is provided by several recent reports (51-53). They reveal that induction of tryptophan pyrrolase and tyrosine χ-ketoglutarate transaminase is blocked by actinomycin D, an inhibitor of RNA synthesis (54-57), but not by ionizing irradiation.

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