Noncytokinetic Radiation Injury: Anticoagulants as Radioprotective Agents in Experimental Radiation Hepatitis

Abstract. *Rats that have been irradiated with a single dose of 1500 roentgens directed to the liver show the following abnormalities: increased clearance of colloidal gold by the liver at 30 days, a decreased albumin space index at 30 days, and increased alkaline phosphatase at 60 days. In each of these cases, animals receiving Depo-heparin in dose ranges reasonable for human application had essentially normal values. There was no radioprotective effect with salicylates at the doses used. Higher doses of salicylates resulted in death of the animals.*

Radiation injury to the human liver, whether all or part has been irradiated, is a form of veno-occlusive disease. Ogata *et al.* first suggested that radiation had a damaging influence on hepatic vessels, especially on the small branches (*1*). Reed and Cox, in their studies of human livers after irradiation, have described early hyperemia, progressive fibrous obliteration of the small branches of the veins, and later loss of cells, particularly near the lobule centers (*2*). Reed and Cox and others postulated that ionizing radiation initiates endothelial damage of small vessels, which in turn leads to a type of veno-occlusive disease. The exact mechanism of this endothelial alteration, especially in subclinical radiation hepatitis, has not yet been completely defined.

The common occurrence of clinically significant radiation hepatitis after doses of only 3000 to 4000 rads seriously limits the use of radiation therapy in the treatment of primary hepatic malignant neoplasm or of metastic tumor of the liver. An interest and concern for the clinical problem of radiation injury to the liver and possible ways to prevent it led us to study experimental radiation hepatitis in rats. When the endothelium of a vessel is injured sufficiently to expose the subendothelial basement membrane or connective tissue, platelets accumulate at the point of injury. Thereafter follows a chain of events that results in a fibrin thrombus. Animal experiments in vivo on cardiac circulation have shown that transient platelet aggregates, even without thrombus formation, can produce microcirculatory disturbances resulting in cardiac dysfunction and tissue injury (*3*). In an attempt to limit platelet aggregation in liver injured by radiation we studied salicylate and heparin as potential radioprotective agents. Heparin affects the coagulation of blood in several ways and decreases platelet stickness as well (*4*).

We used white female National Laboratory rats (150 to 175 g), 7 to 8 weeks old, of Wistar origin. The animals were maintained on standard rat chow and all were housed under similar conditions. The animals (137 in all) were divided into six groups and treated as follows: group A, salicylates only (25); group B, heparin only (25); group C, radiation only (25); group D, radiation plus heparin (25); group E, radiation plus salicylates (25); control group, no treatment (12). From each group 11 or 12 animals were studied 30 days after hepatic irradiation; the remainder of each group was studied at 60 days.

The x-irradiation to the liver was administered with a Westinghouse x-ray therapy unit (220 kv, 15 ma with ½ mm copper and 1 mm aluminum filtration). The therapy unit was calibrated daily with a Landsverk roentgen meter. The animals were anesthetized with 5 to 6 mg of pentobarbital given intraperitoneally. The animals were treated in pairs. Port films of the treatment area were obtained with a double exposure technique, and any errors in port location were corrected before treatment. A single exposure of 1500 roentgens was delivered to the liver of each rat in a radiation study group.

Animals in groups A and E were given sodium salicylate in their drinking water from 1 day before treatment until the time that they were killed. The rats consumed approximately 0.03 mg of salicylates per gram of body weight per day. Animals in groups B and D received Depo-heparin sodium (Upjohn) in the therapeutic range recommended for humans, 200 units per pound of body weight in one injection daily. Injections were begun 1 day before the irradiation and were continued until the animals were killed. Clotting times were checked weekly from tail-vein blood. Clotting times for normal rats ranged from 12 to 30 minutes. Clotting times in the heparinized rats were consistently between 90 minutes and 4 hours. Body weights were recorded weekly.

The 10-minute test for clearance of injected colloidal ^{198}Au was made at 30 and 60 days after irradiations and was used as a measure of reticuloendothelial cell activity in the liver. The ^{198}Au clearance was expressed as the percentage of the injected dose of ^{198}Au per gram of liver. The ^{131}I-albumin 10-minute space was determined at 30 and 60 days and was expressed as ^{131}I (count/min) per gram of liver divided by ^{131}I (count/min) per gram of plasma.

On the day of killing weighed portions of colloidal ^{198}Au and human serum ^{131}I-albumin were injected into the saphenous vein of the rats, which were given light ether anesthesia. Ten minutes later blood samples were withdrawn from the jugular vein, and the animals were frozen rapidly in liquid nitrogen. The animals were dissected in the frozen state and the livers were removed and placed in previously weighed vials. A portion of each liver was fixed for histologic examination. Blood, tissue, and standard samples were counted in a Picker Liquimat. Serum alkaline phosphatase was determined colorimetrically (reagents from Sigma Chemi-

Table 1. Clinical status of rats after irradiation with 1500 roentgens administered as a single dose. The colloid clearance and the albumin space tests were made 30 days after the irradiation. Serum alkaline phosphatase was determined at 60 days. All values are means ± 1 S.E.

Group	Colloid clearance (% injected dose per gram liver)	^{131}I-albumin space ($\times 10^2$)	Alkaline phosphatase (units/ml)
Normal	7.68 ± 0.56	16.20 ± 1.25	3.93 ± 0.31
X-ray	12.41 ± 0.63	12.38 ± 0.77	6.91 ± 0.43
X-ray and heparin	8.96 ± 0.64	15.74 ± 1.04	5.18 ± 0.63
X-ray and salicylates	14.56 ± 0.88	11.06 ± 0.32	6.04 ± 0.39
Heparin only	9.59 ± 0.61	13.66 ± 0.99	5.83 ± 0.24
Salicylates only	14.28 ± 0.69	12.62 ± 0.51	4.84 ± 0.27

cal Company) with results recorded as Sigma units per milliliter at 30 and 60 days, as an indicator of hepatic parenchymal cell injury. The Student *t*-test was applied to the results.

None of the animals developed clinical gastrointestinal manifestations of radiation sickness. At both 30 and 60 days there were only minor differences in the weights of the livers of the various groups either in grams of liver or grams of liver per kilogram of body weight. Compared to the other groups, the animals on oral salicylates tended to gain less weight. Ascites was not present in any animals at the time that they were killed and autopsied. At 30 days the 10-minute, hepatic albumin space was decreased in the irradiated animals (Table 1). As judged by this test, heparin seemed to have a radioprotective effect. The values for the animals treated with x-ray only were significantly different at the $P < .05$ level from those of animals treated with x-ray and heparin. Salicylates showed no radioprotective effect. At 60 days the albumin spaces were not significantly different from those of the controls.

At 30 days the animals treated with x-ray only had an increased colloid clearance (Table 1). The difference between the animals treated with x-ray only and those treated with both x-ray and heparin was significant at $P < .05$. At 60 days both groups had a normal colloid clearance.

There are essentially no differences in the serum alkaline phosphatase at 30 days. At 60 days the alkaline phosphatase of the animals treated with x-ray only was elevated (Table 1), whereas that of animals treated with x-ray plus heparin was close to normal, the difference being significant at $P < .1$. Salicylates, here again, do not show a protective effect. In retrospect, these negative results might have been expected, because salicylates, unlike acetyl salicylic acid, have relatively little effect on blood coagulation in man (*5*).

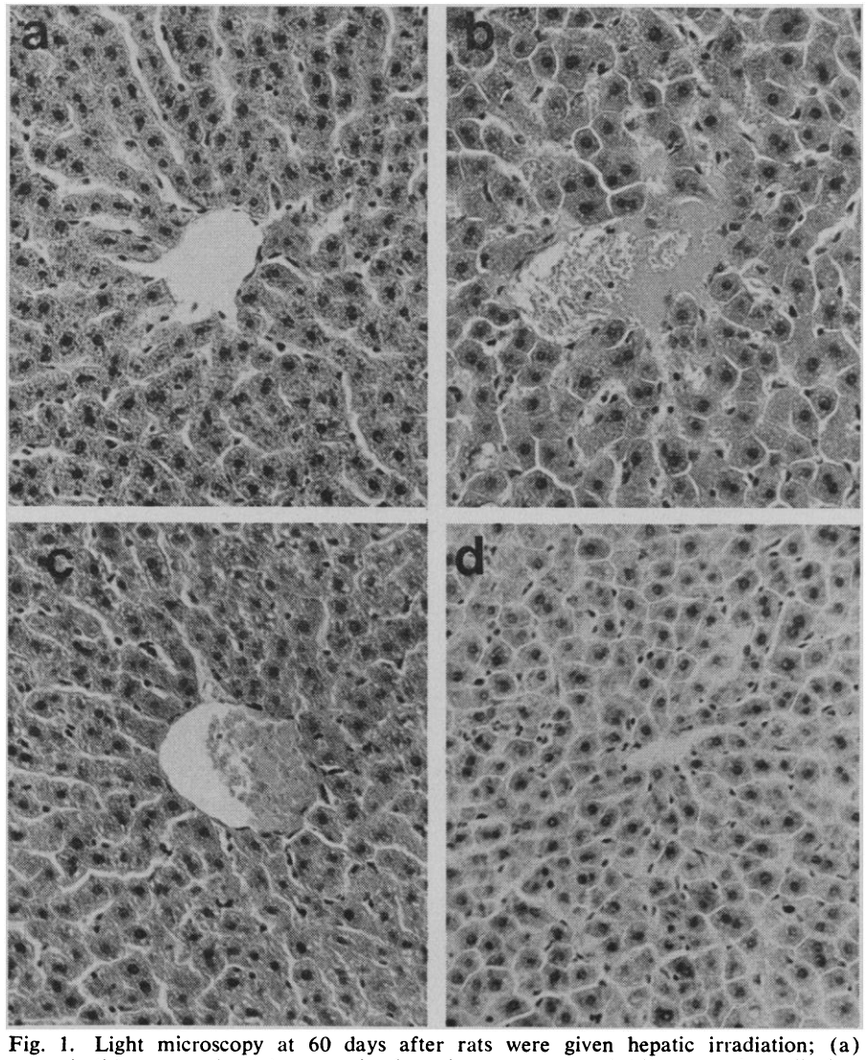

Fig. 1. Light microscopy at 60 days after rats were given hepatic irradiation; (a) control; (b) x-ray only; (c) x-ray plus heparin treatment; and (d) x-ray plus salicylate treatment (× 250).

Light microscopy of liver sections reveals that control, heparin only, and salicylate only groups are histologically similar (Fig. 1a). The liver cells are arranged in columns separated by sinusoids with little space between the cells of any one column. The cells have fine vacuoles and uniform nuclei. The livers of the rats given x-ray only lose somewhat the columnar arrangement and look more like a plate of large polygonal cells with eosinophilic cytoplasm, large cytoplasmic vacuoles, and nuclear pleomorphism (Fig. 1b). In addition to the changes of the individual cells, there are multiple foci of nonspecific inflammation around some central veins and fibrin-like material within the central veins. These changes appear similar to what has been called early thrombus by other investigators (*1, 2*). In rats receiving irradiation and heparin, the structure of the liver more closely resembles that of normal liver with columns and fine vacuoles beginning to reappear (Fig. 1c). In animals receiving irradiation and orally administered salicylates, the damage is similar to that in radiation alone animals except that there is more interstitial inflammation, not only around central veins, but also throughout the hepatic lobules (Fig. 1d). The hepatocytes in the animals treated with both x-ray and salicylate are large and polygonal, with large vacuoles like those in the animals treated with x-ray alone.

In 1965, Ingold *et al.* (*6*) reported that alkaline phosphatase determination had been their single most reliable laboratory index or radiation hepatitis in man. The effect of irradiation on colloid clearance is less well understood. There has been diversity of opinion concerning the functional capacity of the reticuloendothelial system after irradiation. Decreased, normal, and increased function of the Kupffer cells after irradiation has been described (*3, 7–10*). Usually, normal or increased reticuloendothelial function has been found with total body irradiation of less than 1200 roentgens. Our data indicating a decreased albumin space index after a single dose of 1500 roentgens are consistent with that reported by Sassen and his associates (*11*). They measured the vascular and extravascular albumin of eight tissues in mice, including liver, that had been exposed to 800 roentgens of total body irradiation and found a decrease in the total and extravascular albumin (*11*).

Normal microvascular endothelium

does not replicate (that is, there is no evidence of uptake of tritiated thymidine). It seems probable that the radiation injury that responds to anticoagulant therapy is endothelial "damage" and is not dependent on the radiosensitivity or cell-cycle phase of the hepatocytes. Our data support the contention that clinically significant radiation injury does not have to have a cytokinetic basis. The manifestation of radiation damage due to microvascular endothelium injury may be diminished by appropriate systemic anticoagulant therapy.

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Griseofulvin: A Teratogenic Study

Abstract. *Griseofulvin, a fungistatic agent, was administered in oral doses of 125 to 1500 milligrams per kilogram per day to pregnant rats during organogenesis. Evaluation of the offspring from dams treated with the largest doses, 63 and 75 times a therapeutic dose in man, indicated decreased survival rates and a syndrome of malformations.*

Though much is known about griseofulvin's biological actions, its effects on cell division and embryonic development have not been clarified. An intravenous injection of griseofulvin (200 mg/kg), to rats caused disturbances in cell division and maturation in the germinal epithelium of the testes. Mitosis of rapidly dividing cells was transiently arrested at metaphase within 7 hours after administration. Two weeks later testicular histology appeared to be normal (*1, 2*). The fungistatic action of griseofulvin may be due to an interference of nucleic acid synthesis (*3*). Since compounds which block such synthesis have caused teratogenic changes in mammals (*4*), griseofulvin may have teratogenic potential (*1–5*). To investigate this possibility, we performed a reproduction study, using griseofulvin in mature rats.

Groups of 23 to 34 female rats (CAW : CFE SD spf strain) were given either 125, 250, 750, 1250, or 1500 mg/kg per day (*6*). The females were mated with males of proven fertility and then treated orally, from day 6 through 15 after mating, with microsize particles of griseofulvin (*7*) suspended in Tween 80. (Day 0 was defined as the day spermatozoa were detected in the vaginal smear.) On day 21, the dams were killed with an overdose of chloroform, and their offspring were removed. The number of nidation sites and resorptions in each dam was recorded. Approximately one-third of the offspring were examined for soft tissue defects (*8*). The remaining offspring were stained with Alizarin red-S and examined for skeletal defects (*9*).

Dams given 1250 or 1500 mg/kg per day had significantly more resorptions than did the control dams ($P < .01$) (Table 1). The incidence of resorptions, as a percentage of nidation sites, was 5 percent in the controls but 16 percent in the dams given 1250 mg/kg and 64 percent in the dams given 1500 mg/kg. Most resorptions occurred early in pregnancy. The greater number of resorptions in the dams given 1500 mg/kg resulted in a significantly smaller ($P < .01$) average litter size as compared to that in the control group (Table 1).

The body weights of offspring from all groups, except those given 250 mg/kg, were significantly less ($P < .01$) than weights of the controls (Table 1). The weights of the dams given 1250 and 1500 mg/kg were below those of the controls by 13 and 50 percent, respectively.

Skeletal ossification at delivery was retarded about 24 and 48 hours in the offspring of dams given 1250 and 1500 mg/kg, respectively. Offspring growth was commonly reduced in similar studies of other agents (*10*).

Survival rates of the newborns were significantly reduced in all groups, except the one in which the dams were given 250 mg/kg (Table 1). This reduction was related to the dose and was most pronounced in the 1500 mg/kg group where all offspring died within 1 hour after birth.

Somal examination revealed that abnormalities occurred in some pups from four of the treated groups but not in the controls (Table 2). Specifically, one pup from a dam given 250 mg/kg had clubbed feet, no eyes, one gonad, anal atresia, angulated ribs, and deformed skull bones. One pup from a dam given 750 mg/kg had dilated renal pelves. Nineteen of the 222 pups (8 percent) from dams given 1250 mg/kg had tail anomalies (no tails, or shortened or kinky tails), and one pup had exencenphaly. Nine of 90 (10 percent) of the pups from dams given 1500 mg/kg had tail deformities, and one of these pups also had exencephaly.

Table 1. Reproduction data. Rats were treated with griseofulvin daily from day 6 through day 15 after mating. Pups were weighed individually, and the weights were averaged by dose group. Data from treatment groups were compared with data from control groups. Survival rates were determined as follows: (number of pups surviving for 24 hours/number of pups retained for 24 hours) × 100.

Dose per day (mg/kg)	Rats				Litter size*	Pup weight*	24-hour survival rate (%)
	Pregnant rats (No.)	Nidations (No.)	Resorptions (No.)	Resorptions (%)			
None	19	278	14	5.0	13.9 ± 0.7	5.4 ± 0.02	100
125	14	187	3	1.5	13.6 ± 0.7	5.1 ± 0.03†	94
250	19	236	5	2.1	12.2 ± 0.8	5.5 ± 0.02	100
750	24	310	8	2.6	12.6 ± 0.3	5.1 ± 0.03†	93
1250	19	290	48	16.5†	12.7 ± 0.5	4.7 ± 0.04†	80
1500	12	252	162	64.3†	7.5 ± 1.3†	2.4 ± 0.08†	0

* Result is given as the mean ± the standard error of the mean. † Significant differences ($P < .01$) are indicated by a dagger.