matter produced a mean decrease of 3.2 mg of cholesterol/100 ml of serum. Although this effect is statistically non-significant, it matches the discrepancy observed in the earlier comparisons. It is concluded that the unsaponifiable matter of 100 g of corn oil lowers serum cholesterol by something between 6 and 12 mg 100 ml.

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Failure to Demonstrate Toxic Factors in the Serum of Irradiated Rats.* (24070)

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Although circulating toxic humoral factors may play a role in radiation-induced illness, little unequivocal evidence supports such a factor(1). It has been demonstrated that serum from irradiated rabbits, incorporated into the media for growing chick heart fibroblasts and *Escherichia coli* depressed their rate of cell division(2). Van Dyke and Huff(3) reported that, in rats parabiotically united before irradiation of one of the parabionts with 900 r of X-radiation, epilation of both parabionts occurred. Non-irradiated parabiotic rats and single rats given 900 r did not show this effect. Lavik *et al.*(4), assumed that toxic factors were dialyzable but was not able to prolong, by *in vivo* dialysis, the lives of dogs which were given 500 r of whole body X-radiation. Whole blood taken at various time intervals post-irradiation from rats given supralethal amounts of whole body Co^{60} gamma radiation failed to cause weight changes or mortality in rats injected intravenously with this material(5). Recently Edelmann presented evidence indicating existence of lethal toxic substances in blood of adrenalectomized rats.‡ He reported that adrenalectomized rats and mice, injected intraperitoneally and intravenously with serum, plasma, and whole blood from other adrenalectomized irradiated rats (1000 r) died sooner than adrenalectomized animals which received blood from non-irradiated adrenalectomized donors.

The present experiments were initially de-

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signed to investigate physiological and histological effects of serum from irradiated animals. Before attempting to do this, serum from irradiated adrenalectomized rats was assayed along lines similar to those used by Edelmann. This report is concerned with results of this and other assay procedures. Since a toxic effect was not detected, histologic studies were not performed.

Materials and methods. Male and female Sprague-Dawley rat donors were adrenalectomized 1 or 2 days prior to irradiation and maintained on saline (1% NaCl) until irradiation and exsanguination. Sprague-Dawley rats and Carworth Farm recipient mice were adrenalectomized 1 or 2 days prior to injection. Adrenalectomy in all cases was performed through paravertebral lumbar incisions under sodium pentobarbital or ether anesthesia. Care was taken to remove the adrenal pedicle with a maximal amount of surrounding fat without damage to the capsule. Bleeding was absent or insignificant. The donors were irradiated with X- or gamma radiation 24 hours prior to exsanguination. Radiation factors were as follows: radiation was delivered by General Electric Maxitron therapy unit at 250 KVP; 30 ma; 0.5 mm Cu, 1 mm Al filter; HVl 1.5 mm Cu; TSD 70 cm. Tissue dose measurements were made with Victoreen chamber placed in box containing the animals; the tissue dose so measured was 70.4 r/minute. Gamma irradiation procedure has been described(6). Donors were given total body radiation of either 1000 r X-radiation or 1300 r gamma radiation. Exsanguination procedure was as follows: donor rats were anesthetized with ether until respiratory arrest; the abdominal cavity was opened and blood withdrawn from abdominal vena cava through a #20 hypodermic needle. A dry 10 cc syringe was used for each exsanguination. The blood was transferred to a dry glass centrifuge tube and allowed to clot for 30 minutes at 0-5°C. The clotted material was centrifuged at 1500 rpm at 2°C for approximately 30 minutes. The serum thus obtained was kept at 0-5°C until injection. All injections were made within one to 3 hours post-exsanguination. Intravenous injections were made via tail veins using #27 gauge needle. In all experiments involving adrenalectomized animals, pyrogen-free glassware and needles were employed for exsanguinations, blood processing, and injections. Pyrogen-free equipment was prepared in an oven at 170-180°C for 2 hours. Another type of assay involved degree of spleen-thymus weight loss, responsive to doses of radiation well below the lethal range(7). Three hundred and sixty intact female mice, 4 to 6 weeks old, were injected intravenously with serum from irradiated (1000 and 5000 r) and non-irradiated rats in doses of 0.25, 0.50, and 0.75 ml. There were 120 untreated control animals. Approximately 80 hours post-injection, the mice were sacrificed and spleens and thymi removed. Spleens were weighed on Roller-Smith torsion balance. Thymi were fixed in Carnoy's fixative, cleaned, and blotted semi-dry before weighing. In third type of assay, 650 r of Co^{60} gamma radiation was given to 90 mature, intact male CF-1 mice. Thirty of these received intravenously 0.6 ml of serum from adrenalectomized male and female rats which had been given 1300 r of Co^{60} gamma radiation 24 hours previously in attempt to increase mortality. One control group of 30 irradiated mice received the same amount of serum from non-irradiated adrenalectomized donors. Another control group of 30 was not injected. The mice were injected on seventh post-irradiation day. Observations for mortality were made daily for a 30-day period. Another group of recipient mice was given LD 22/30 dose (640 r) of gamma radiation and injected intravenously on 1st, 2nd, 3rd, and 4th post-irradiation day with 0.5 ml of serum from intact rat donors given 1000 r whole body X-radiation 1, 2, 3, and 4 days prior to bleeding.

Results. Fig. 1 represents results of 4 replicate experiments in which a total of 95 adrenalectomized rat recipients received intravenously 0.5 ml of serum from X-irradiated donors. Eighty-eight rats received serum from non-irradiated adrenalectomized donors and 69 others were not injected. Female donors and recipients were used; recipients weighed 40 to 80 g. There was no significant increase in mortality in group which received serum from irradiated donors. The apparent dif-

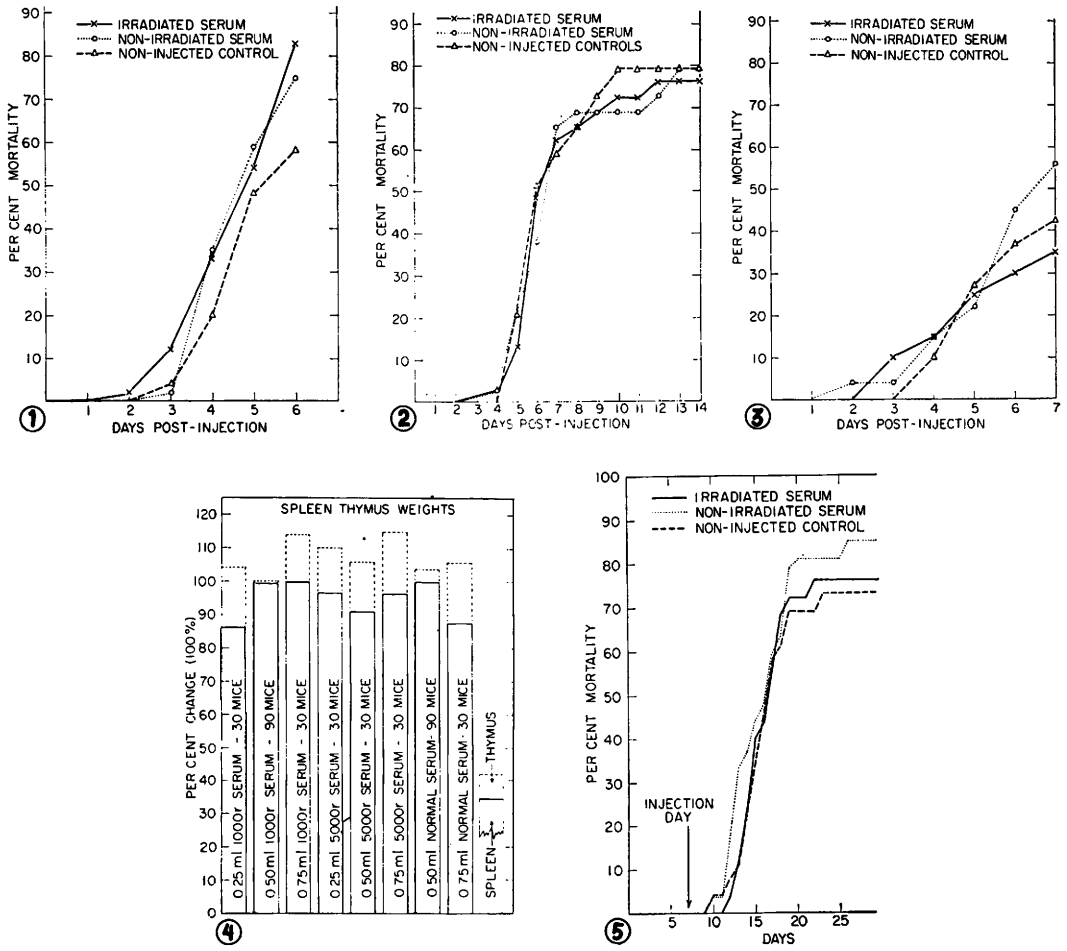

FIG. 1. Effect of intrav. administered serum (0.5 ml) from irradiated and non-irradiated adrenalectomized rats in adrenalectomized rat recipients.

FIG. 2. Effect of 4 ml of serum from adrenalectomized irradiated and non-irradiated rats administered intraper. to adrenalectomized rats.

FIG. 3. Effect of 0.6 ml of intrav. administered serum from irradiated and non-irradiated adrenalectomized rats in adrenalectomized mice.

FIG. 4. Spleen-thymus assay.

FIG. 5. Mortality effect of serum from adrenalectomized rats administered intrav. to pre-irradiated mice.

ference on third post-injection day is statistically insignificant.

Twenty-nine adrenalectomized female rats weighing 70 to 100 g and receiving intraperitoneally 4 ml of serum from other irradiated (1300 r gamma) adrenalectomized rats died at same rate as did 29 adrenalectomized rats which received 4 ml of serum from non-irradiated adrenalectomized donors and 29 non-injected adrenalectomized rats (Fig. 2).

There are no significant mortality differences in adrenalectomized mice which received 0.6 ml of serum from irradiated adrenalectomized rats and those which received the same amount of serum from non-irradiated donors or those which were not injected (Fig. 3). There were 30 animals in each group. Another experiment similar to this one, except that donors were adrenalectomized 48 hours prior to irradiation instead of 24 hours, gave same negative results.

The results of spleen-thymus assay are presented in Fig. 4. There was no significant atrophy of spleen or thymus after intravenous administration of serum from intact irradiated rats. Per cent change is measured from

100%, established on the basis of 120 non-injected control animals.

Fig. 5 shows that there was no potentiation of the effects of 650 r gamma radiation in mice by injection of serum taken from irradiated rats. This last type of experiment showed that there were no differences in mortality rate in pre-irradiated mice which received serum from irradiated rats during 24 hours to 96 hours post-irradiation. There was a minimum of 30 mice in each recipient group.

Discussion. The results of these experiments were negative. However, they signify little unless a reasonable estimate of sensitivity of the assay procedures can be determined, *i.e.,* are they sufficiently sensitive to give a qualitative indication of presence of radiotoxic material? To answer this question adequately, 2 assumptions must be made, (1) that toxic substances, if produced, would be carried in blood of irradiated animals and (2) that at some time after irradiation there would be sufficient toxic material in the circulating humor to produce observable effects in other animals. Since all blood and hence all toxic material will not be obtained and administered by ordinary exsanguination and injection procedures, a recipient test animal which is considerably more sensitive than normal should improve chances of detection.

It would seem likely that sensitizing an animal with lethal doses of radiation would make the animal more susceptible to deleterious effects of added radiotoxic material. Noble estimated that the threshold of response to any noxious agent by adrenalectomized animals is 5 to 20 times below that of their normal counterparts(8). Spleen and thymus involution resulting from very low doses of radiation would also appear to be a good biological criterion for measuring radiotoxicity of serum(7). These 3 sensitivity assays should be able to detect small amounts of toxic substances. Since no effect was detected, the hypothetical toxic substances were either in very low concentrations, slightly toxic or non-existent. At any rate these "radiotoxins" would appear of very little importance in the acute radiation syndromes. It is probable that large doses of radiation may result in liberation of physiologic or other substances capable of producing some transient and diverse physiological changes, particularly cardiovascular, following heavy exposure. It is clear from present experiments, however, that the hypoplasia and its sequelae seen with the syndrome of acute radiation injury is not mediated through liberation of a humoral toxic substance. The above considerations do not exhaust all possible alternatives. If toxic factors are extremely labile, they might be inactivated by handling. Associating sickness with toxic substances is not always apparently valid, *e. g.,* in avitaminosis. It is possible that an analogous situation exists in the irradiated animal. Toxic factors might be metabolized too rapidly for any detectable amounts to accumulate or they may diffuse out of the capillaries at a particular extravascular locus of action so that their presence in the blood would be of a very evanescent nature. Toxic factors might then be detectable at such locations or at a particular site of elaboration.

Summary. Three experimental procedures were utilized to assay serum from irradiated rats for radiotoxic substances. Such toxic material was not demonstrable by these technics. These experiments do not preclude the existence of radiotoxins in the irradiated animal, but they do indicate that if radiotoxins exist, the blood is probably not their principal storehouse. If, on the other hand, the blood does figure significantly in their storage, then they are not responsible for the severe signs and symptoms resulting from acute total body exposure.

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