A Comparision of Phospholipid Biosynthesis in Irradiated Bone Marrow and Liver. (31950)

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One of the important effects of radiation on animals is the increase in the number of cells that contain triglycerides in the bone marrow at a time when the blood cell precursors are decreased in number(1). The stimulation of triglyceride biosynthesis in the marrow by irradiation occurs in a non-fat cell fraction of marrow(2) and only at marrow sites directly exposed to irradiation(3).

The metabolism of the individual phospholipids of bone marrow has not previously been investigated during the period when triglyceride biosynthesis becomes the main pathway for fatty acid metabolism. This communication describes the comparison of phospholipid pool sizes and the incorporation of labeled fatty acids into phospholipids of irradiated bone marrow and liver.

Materials and methods. All animals were lightly anesthetized with diethyl ether for oral intubation of 1-^{14}C fatty acids in corn oil (20 μc per 100 g of body weight). Radiopurity of the 1-^{14}C palmitic and 1-^{14}C oleic acids (New England Nuclear Corp.) was 98% and 95%, respectively, based on zonal profile scans(4) of thin-layer chromatograms (TLC). Sixteen Carworth Farms Nelson's strain female rats weighing approximately 150-200 g each were used in this study. Eight animals were exposed to 800 r total-body irradiation (4.43 r/min air dose) from 8 cesium-137 sources(1) 4 days before sacrifice. After light anesthesia, we decapitated irradiated (4 days after 800 r) and control groups at 2, 4, 6, and 12 hours after administering fatty acids.

Blood was drained as completely as possible before a portion of the liver and both femurs were removed from the animal. The serum was subsequently separated by centrifugation at 2000 $\times$ *g*. Liver tissue was homogenized in an all-glass Potter Elvehjem homogenizer, whereas marrow from the femurs was extruded directly into glass vials using nitrogen pressure. All tissues were lyophilized before lipid extraction(5) with chloroform: methanol (2:1 v/v). All lipid extracts were dried in a rotary vacuum evaporator or under a stream of dry nitrogen. The lipid residue was redissolved in chloroform for TLC.

Skipski *et al*(6) developed the procedure we modified slightly for the TLC separation of phospholipids. This chromatographic system does not distinguish the alkoxy and alkenyl ether-containing phospholipids from the corresponding ester-containing phospholipids, nor does it resolve phosphatidyl inositol. Known concentrations of total lipids or standards (Applied Science Laboratories) in chloroform (10-30 mg/ml) were spotted on Silica Gel HR (Brinkmann Instruments, Inc.) layers that had been activated for 30-60 minutes at 110°C. The silica gel slurry was prepared in 0.01 M Na_2CO_3 solution to make the layers basic. The chromatoplates were developed in fully-lined saturated chambers containing cloroform:methanol:glacial acetic acid : saline (50:25:8:4 v/v/v/v). The saline provided excess ions, thereby reducing the intermolecular electrostatic interaction of the highly polar phosphatides(7) and improving resolution. The chromatoplates were developed a distance of 14 cm, and the resolved phospholipids were visualized by exposure to iodine vapor or by heating in an oven at 180-200°C after spraying with concentrated H_2SO_4. Zones representing lecithin, cephalin, phosphatidyl serine, lysolecithin, sphingomyelin, and the neutral lipids were scraped with a razor blade by hand into liquid scintillation vials for radioassay (4), using a toluene, naphthalene, methylcellosolve water solvent system with BBOT (2,5

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bis [2-(5-*tert*-butylbenzoxazoly)]-thiophene) as scintillator(8). Photodensitometry was done according to the quantitative charring procedure of Privett and Blank(9). Identification of the phospholipid zones was based solely on standards that were cochromatographed on adjacent TLC lanes. Phospholipid phosphorus before and after TLC was also measured(10).

TABLE I. Effect of Total Body Irradiation on Phospholipids of Marrow and Liver.

	Control*		4 days after 800 r*	
	Marrow	Liver	Marrow	Liver
Lipid P				
μg/mg lipid	6.02 ± 1.79	19.78 ± 1.53	1.52 ± 0.17	22.21 ± 1.84
μg/mg wet wt	.34 ± .04	.68 ± .11	.15 ± .01	.70 ± .10
μg/mg dry wt	1.28 ± .29	2.06 ± .59	.54 ± .03	2.38 ± .32
Lecithin P				
μg/mg lipid	3.01 ± 0.90	10.12 ± 1.22	0.86 ± 0.14	12.25 ± 1.15
μg/mg wet wt	.18 ± .01	.34 ± .04	.08 ± .01	0.39 ± .04
μg/mg dry wt	.66 ± .08	1.05 ± .27	.31 ± .01	1.31 ± .10
Cephalin P				
μg/mg lipid	1.81 ± 0.50	5.78 ± 0.73	0.43 ± 0.16	6.06 ± 0.43
μg/mg wet wt	.11 ± .01	.20 ± .04	.04 ± .01	.19 ± .02
μg/mg dry wt	.40 ± .06	.60 ± .19	.15 ± .05	.65 ± .07

* Based on duplicate samples from 4 rats per group.
The numbers preceded by ± are one standard deviation from the mean value.

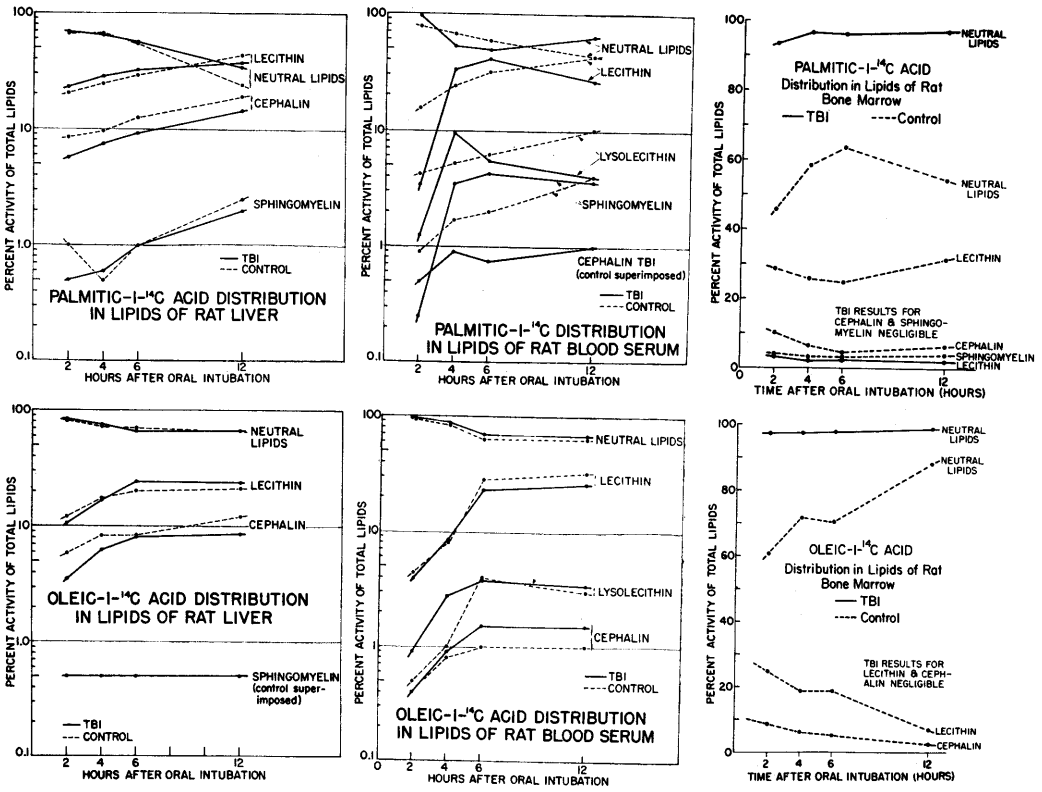

FIG. 1. Incorporation of orally administered 1-^{14}C-labeled palmitic or oleic acids into phospholipids of liver, serum, and marrow of control and irradiated rats (4 days after 800 r). The curves for bone marrow were plotted on linear scales, whereas the curves for liver and serum were plotted on semi-log scales so that the wide range of values could be indicated on a single graph.

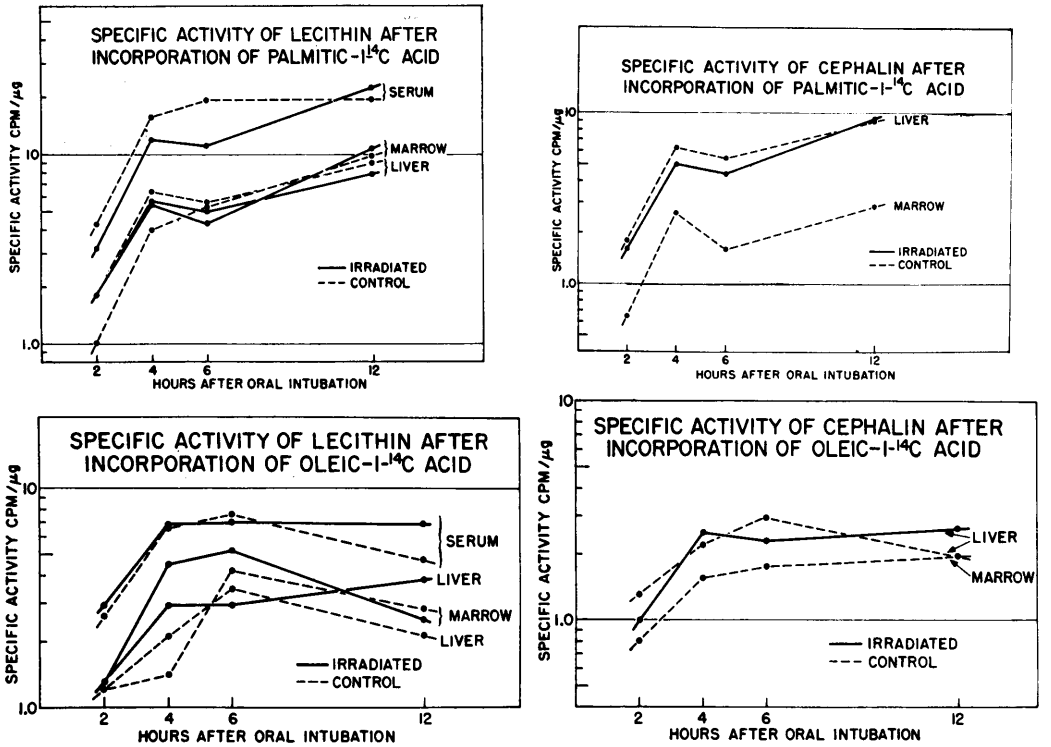

FIG. 2. Specific activity curves for lecithin and cephalin of rat bone marrow after the oral injection of 1-^{14}C-labeled palmitic or oleic acids. Irradiated rats were exposed to 800 r total-body irradiation 4 days earlier.

Results and discussion. As predicted from the shifts in marrow cell population caused by ionizing irradiation, a significant decrease in the bone marrow pool of phospholipids occurred. The decrease in lecithin P and cephalin P (Table I) was commensurate with the decrease in hemopoietic elements(2) 4 days after 800 r. On the other hand, the phospholipid-phosphorus in liver was not altered by irradiation (Table I).

Fig. 1 shows the distribution of 1-^{14}C palmitic and oleic acids into the major lipid classes in serum, liver, and marrow of both control and irradiated rats. The greatest incorporation of the labeled fatty acids occurred in the neutral lipid fraction of the tissues examined. In the phospholipid fractions of all tissues, the incorporation of labeled fatty acids was highest in the lecithin. Total-body irradiation had no significant effect on the incorporation of labeled palmitic and oleic acids into the liver or serum lipids. The lecithin and cephalin of femoral bone marrow, the major phospholipids present in this organ contained most of the ^{14}C present in the phospholipid fraction. However, most of the ^{14}C in the irradiated bone marrow was present as triglyceride, confirming earlier experiments(2,3).

Despite the depressed incorporation (Fig. 1) of label into lecithin and cephalin of irradiated bone marrow, the specific activities (Fig. 2) of lecithin in the marrow were not appreciably changed in either liver or bone marrow after irradiation. There was not enough cephalin present in irradiated bone marrow to calculate specific activity (Fig. 2). Since the specific activities of lecithin in bone marrow were unaltered by irradiation in spite of a great decrease in the lecithin pool, we conclude that lecithin formation remains unchanged in those hemopoietic cells that are still functional in the injured marrow.

Summary. Total phospholipid phosphorus, lecithin P, and cephalin P of bone marrow, but not of liver, were markedly decreased in rats 4 days after total-body irradiation. Ex-

periments measuring the incorporation of palmitic-1-^{14}C and oleic-1-^{14}C acids into various phospholipids of blood serum, liver, and femoral bone marrow of normal and irradiated rats revealed that the lecithin was the major phospholipid incorporating radioactivity in all tissues. Total-body irradiation did not appear to exert any appreciable effect on the specific activities of these phospholipids in any of the tissues studied. Although radioactivity distribution studies showed little change in liver and serum, radiation significantly decreased the amounts of both palmitic-1-^{14}C and oleic-1-^{14}C acids incorporated into bone-marrow phospholipids. The data are explained on the basis of changes in the marrow-cell population occurring after irradiation.

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Fetal Liver: A Source of Immunoglobulin Producing Cells in the Mouse.* (31951)

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It has been shown in studies of the ontogeny of the mouse immune system that the cells which participate in "delayed hypersensitivity" and homograft reactions are derived from fetal liver(1-3), and that these cells are dependent upon the thymus for their functional maturation(4). The ontogenesis of the cells responsible for antibody production in the mammal is not known, although it has been suggested that the appendix or Peyer's patch-type structures may serve as the source of these cells(5). This idea finds support in work with the chick which shows that the bursa of Fabricius, a gut associated lymphoepithelioid structure, is critically involved in the development and regulation of antibody forming cells(6-8). On the other hand, studies in the mouse suggest that antibody forming cells, regardless of their site of origin, are dependent upon intact thymic function for their functional maturation or proliferation or both(9-12). It was felt, therefore, that further work was needed before definite conclusions could be drawn with regard to the origin and regulation of mammalian antibody forming cells. In this report, data are presented which demonstrate that cells from fetal liver are capable of producing immunoglobulins in thymectomized as well as in intact hosts.

Materials and methods. Twelve-week-old thymectomized and nonoperated (C57L $\times$ A)F_1 mice received 870 rad whole body X radiation, and immediately thereafter they were given an intraperitoneal injection of 33 $\times$ 10^6 nucleated cells derived from the livers of 17 day C57Bl/6 $\times$ C57Bl/6 embryos (care was taken to exclude gut or gut associated structures from the sample). These strains were chosen because of their independently determined gamma-globulin allo-

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