

periments measuring the incorporation of palmitic-1-¹⁴C and oleic-1-¹⁴C 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-1-¹⁴C and oleic-1-¹⁴C acids incorporated into bone-marrow phospholipids. The data are explained on the basis of changes in the marrow-cell population occurring after irradiation.

The authors express thanks to Mrs. Anita Moehl for technical assistance.

1. Snyder, F., Cress, E. A., *Rad. Res.*, 1963, v19, 129.
2. Snyder, F., *ibid.*, 1966, v27, 375.
3. Snyder, F., Wright, R., *ibid.*, 1965, v25, 417.
4. Snyder, F., *Anal. Biochem.*, 1964, v9, 183.
5. Folch, J., Lees, M., Sloane Stanley, G. H., *J. Biol. Chem.*, 1957, v226, 497.
6. Skipski, V. P., Peterson, R. F., Sanders, J., Barclay, M., *J. Lipid Res.*, 1963, v4, 227.
7. Marinetti, G. V., *ibid.*, 1962, v3, 1.
8. Snyder, F., Smith, D., *Separation Sci.*, 1966, v1, 709.
9. Privett, O. S., Blank, M. L., *J. Am. Oil Chem. Soc.*, 1962, v39, 520.
10. Rouser, G., Siakotas, A. N., Fleischer, S., *Lipids*, 1966, v1, 85.

Received December 1, 1966. P.S.E.B.M., 1967, v124.

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

M. L. TYAN, L. J. COLE, AND L. A. HERZENBERG

U. S. Naval Radiological Defense Laboratory, San Francisco and Department of Genetics, Stanford Medical School, Palo Alto, Calif.

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 lympho-epithelioid 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 × A)F₁ mice received 870 rad whole body X radiation, and immediately thereafter they were given an intraperitoneal injection of 33 × 10⁶ nucleated cells derived from the livers of 17 day C57Bl/6 × 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-

* Supported in part by funds from Bureau of Medicine and Surgery, U. S. Navy; and USPHS grants CA-04681 and GM-12075.

TABLE I. Production of Gamma-2a Globulins by C57Bl/6 Fetal Liver Cells in Lethally Irradiated Thymectomized and Nonoperated (C57L × A)_F₁ Mice.

Thymectomy	Mouse No.	Gamma-globulin allotype levels in host sera*			
		(Days after radiation)			
		30	45	58	Host
		Donor	Donor	Donor	Host
No	1	1+	2+	3+ (20)	4+ (21)
	2	—	1+	1+ (8)	4+ (30)
	3	1+	2+	2+ (10)	4+ (20)
	4	2+	3+	4+ (20)	3+ (30)
	5	1+	2+	3+ (12)	4+ (20)
Yes	1	±	1+	1+ (8)	3+ (8)
	2	1+	3+	3+ (23)	4+ (9)
	3	1+	3+	4+ (30)	4+ (8)
	4	±	2+	2+ (10)	4+ (18)
	5	—	—	1+ (4)	4+ (18)

* The results are expressed as semi-quantitative estimates (*i.e.*, 0 to 4+). Figures in parentheses represent percentage of specific gamma-globulin allotype present in the serum as compared to that present in the standard reference sera.

types(13). The mice were bled from the retro-orbital plexus 30, 45 and 58 days after irradiation. The sera were assayed for donor type gamma-globulins by a semiquantitative double-diffusion-in-gel method. In addition, the sera obtained 58 days after irradiation were quantitatively tested for both donor and host type gamma-globulins by an inhibition of precipitation assay with I¹²⁵ labelled antigen as described previously(14). Standard serum pools of donor or host allotype were used as reference sera.

Results and discussion. As can be seen in Table I, donor type gamma-globulin was first detected in small quantities 30 days after injection of the donor cells; by 45 days after irradiation both the nonoperated and the *thymectomized* mice had appreciable quantities of donor type gamma-globulin in their sera. At 58 days all the mice had roughly the same levels of donor and host type gamma-globulins. These results demonstrate that cells derived from fetal liver are capable of producing gamma-globulins whether placed in thymectomized or in intact irradiated hosts. As it is known that under these experimental conditions, thymectomized mice produce little or no antibody in response to specific antigenic challenge(9,10), the nature of the donor "immunoglobulins" found in these thymectomized hosts is of great interest. A knowledge of the molecular structure of these globulins and of whether they represent specific anti-

body or immunologically inert proteins might add greatly to our understanding of the mechanism of specific immunoglobulin synthesis and the role played by the thymus in this process. Studies are being conducted in an effort to answer some of these questions.

Summary. Thymectomized and nonoperated adult mice were given a potentially lethal dose of X radiation and protected with allogeneic fetal liver cells. Immunoglobulins of donor as well as host type were found in the sera of the *thymectomized* and intact hosts as early as 30 days after irradiation.

NOTE ADDED IN PROOF:

Additional chimeras have been produced using fetal liver cells from 14-21 day C57Bl/6 × C57Bl/6 embryos. All of these mice, thymectomized (21) as well as intact (10) had significant quantities of donor-type γ -globulins in their sera. These mice were sensitized to a synthetic polypeptide (TGAL) and their sera were tested for total and in three instances for donor-type anti-TGAL antibody. All of the fetal liver cell chimeras (intact thymus) produced specific antibody (10/10), while none of the thymectomized chimeras (0/21) had detectable antibody. Of the three mice tested in the intact-thymus group, from 10% to 75% of the specific antibody was of donor origin.

We wish to express our appreciation to G. M. Iverson and M. Peacock for technical assistance.

1. Tyan, M. L., Cole, L. J., *Transplantation*, 1963, v1, 347.

2. ———, *ibid.*, 1964, v2, 241.
3. Tyan, M. L., Cole, L. J., Nowell, P. C., *ibid.*, 1966, v4, 79.
4. Tyan, M. L., *Science*, 1964, v145, 934.
5. Archer, O. K., Sutherland, E. R., Good, R. A., *Nature*, 1963, v200, 337.
6. Glick, B., Chang, T. S., Jaap, R. G., *Poultry Sci.*, 1956, v35, 224.
7. Warner, N. L., Szenberg, A., *Nature*, 1962, v196, 784.
8. Isakovic, K., Jankovic, B., Popeskovic, L., Milosevic, D., *ibid.*, 1963, v200, 273.
9. Miller, J. F. A. P., *Lancet*, 1961, ii, 748.
10. Tyan, M. L., Cole, L. J., *Transplantation*, 1966, v4, 557.
11. ———, *Clin. Exp. Immunol.*, 1966, v1, 403.
12. ———, *ibid.*, 1967, v2, 121.
13. Herzenberg, L. A., Warner, N. L., Herzenberg, L. A., *J. Exp. Med.*, 1965, v121, 415.
14. Herzenberg, L. A., *Regulation of the Antibody Response*, B. Cinader, Ed., C. C Thomas, Springfield, Ill., 1967.

Received December 1, 1966. P.S.E.B.M., 1967, v124.

Susceptibility of Male and Female Mice to the Nephrotoxic and Hepatotoxic Properties of Chlorinated Hydrocarbons.* (31952)

CURTIS D. KLAASSEN[†] AND GABRIEL L. PLAA

Department of Pharmacology, College of Medicine, University of Iowa, Iowa City

It is well known that a number of chlorinated hydrocarbons cause severe injury to the liver and kidneys. Eschenbrenner(1) reported that after administration of chloroform, renal necrosis occurred in male, but not in female mice. This sex difference has been confirmed (2,3). Culliform and Hewitt(4) have reported that female mice become fully susceptible to necrosis after treatment with androgens, and that the susceptibility of male mice to kidney necrosis is removed by castration and adrenalectomy. Sex differences in the morphology of the mouse kidney have been reported by Crabtree(5), who showed that the parietal layer of most of the Bowman's capsules in female mice is composed entirely of squamous cells, while in most of the capsules in male mice it is composed partly or entirely of cuboidal cells similar to those of the proximal convoluted tubules.

While marked differences exist between male and female mice to the susceptibility of kidney necrosis produced by chloroform, it is generally thought that no difference exists in the susceptibility to liver damage(1,4). However, recently Meshorer and Benhar(6) reported that they observed a difference in sus-

ceptibility to liver damage by carbon tetrachloride in male and female mice.

The purpose of the present work is 1) to determine if hydrocarbons, other than chloroform, also show sex differences in kidney damage, and 2) to determine if a sex difference also exists in the hepatotoxic response to carbon tetrachloride.

Methods. Male Swiss-Webster mice were randomized 10 per cage and used throughout. Two different weights of mice were used, 25-35 g and 35-45 g. The smaller mice were used except where stated otherwise.

Analytical grades of the following hydrocarbons were employed: chloroform; carbon tetrachloride; and 1,1,2-trichloroethane. All agents were administered intraperitoneally and were made up in corn oil to deliver the proper dosage in a final volume of 0.01 ml/g.

Lethality. For the 24-hour LD₅₀ determinations 4 or 5 groups of mice, 10 per group, were injected with a single dose of the hydrocarbon, and the number of deaths recorded at the end of 24 hours. The median lethal dose (LD₅₀) was then calculated for each hydrocarbon.

Kidney function. A urine collection unit for the kidney function phase was used as previously described by Plaa and Larson(7). Urine was collected and tested directly without ad-

* This work was supported by funds from USPHS (Research Grant AM-05802) and by funds from Dow Chemical Co., Midland, Mich.

[†] USPHS predoctoral Fellow (5-F1-GM-30,996).