the other organs (Table I). Fig. 3 illustrates the positive staining of thymic lymphoma cells from a representative far advanced lymphomatous thymus.

Although difficult to illustrate by photographic means, under direct examination with the microscope the stain precipitate appeared in the lymphoma cells as a very fine black particulate deposition localized at the cytoplasmic borders and in occasional tumor cells at the nuclear membrane as well (Fig. 3).

The data in the present study indicate that the increase in alkaline phosphatase activity observed in thymic lymphoma arises only *after* definite lymphoma cells are present in one thymus and does not even appear until these tumor cells have already proliferated sufficiently to enlarge that thymus at least 3× in weight. These data, together with the variability in staining response reported by Metcalf *et al,* and confirmed in this study (Group 4 animals), suggest that the increased level of alkaline phosphatase in lymphoma tissue is a consequence rather than a cause of the neoplasia. This interpretation, based on histochemical evidence, is compatible with that reached by Metcalf *et al,* who concluded that the "alkaline phosphatase levels became elevated in mouse lymphocytes only after the cells become frankly neoplastic". The data from the present study differ from those reported by Lagerlöf and Kaplan(3), who found that the alkaline phosphatase appeared concomitantly with the first morphological signs of tumor development. Possibly differences in the staining techniques, in the host-virus system used, and in histologic interpretation, may explain the lack of agreement of results.

Summary. Mice inoculated with a leukemogenic virus (Rich) were studied to determine when alkaline phosphatase appears in thymic cells relative to the developing thymic lymphoma. The findings indicated that the enzyme increase occurs only after the tumor is definitely established, hence this change cannot be causally related to the neoplasm.

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Effect of Age and Species on Sensitivity of Lymphocytes to Prednisolone and Phytohemagglutinin.* (32227)

KARAM V. BATRA AND ROBERT SCHREK

Tumor Research Laboratory, Research Service, Veterans Administration Hospital, Hines, Ill.

Prednisolone has been shown to have a delayed cytocidal effect on blood and splenic lymphocytes of rats, mice and rabbits. In contrast, lymphocytes from the blood and spleen of men and guinea pigs were found to be relatively resistant to the hormone(1). Similarly the blood lymphocytes of man and lower animals differed in sensitivity to phytohemagglutinin(2). As these reagents produce interesting differential biologic effects, we studied the effects of the reagents on lymphocytes from the thymus, spleen and appendix of neonatal, young and adult rabbits and from the thymus of neonatal man, rat and mouse. Of particular interest was the finding that thymocytes of neonatal rabbits are resistant to prednisolone.

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Methods. The tissues used were the thymus,

TABLE I. Effect of Prednisolone (10 μg/ml) and X-Irradiation (100 r) on Lymphocytes from the Thymus of Human Fetus and Newborn Rabbit, Rat and Mouse Incubated for 24 Hours at 37°C.

	Prednisolone			X-irradiation		
Source of lymphocytes	No. of exp	% Survival of untreated lymphocytes*	% Effect†	No. of exp	% Survival of untreated lymphocytes	% Effect
Rabbit	19	62.4 ± 2.2‡	3.8 ± 1.6	10	60.9 ± 2.5	42.5 ± 3.5
Rat	3	55.5 ± 2.8	98.8 ± .8	4	68.8 ±19.0	88.1 ± 4.8
Mouse	10	54.5 ± 4.2	100	6	63.3 ± 7.2	70.4 ± 5.5
Human fetus	2	39.7	76.9	2	39.7	93.2

$$* \%\ \text{Survival} = 100\left[\frac{\text{Viable lymphocytes/mm}^3\text{ in control incubated suspension}}{\text{Viable lymphocytes/mm}^3\text{ in original suspension before incubation}}\right].$$

$$\dagger \%\ \text{Effect} = 100\left[1-\frac{\text{Viable lymphocytes/mm}^3\text{ in treated incubated suspension}}{\text{Viable lymphocytes/mm}^3\text{ in control incubated suspension}}\right].$$

‡ S.E. of mean.

spleen and appendix of rabbits of various ages and the thymus from newborn rats and mice. In addition, the thymic glands of 2 stillborn human fetuses were tested 24 to 48 hours after excision.

The tissues of the lower animals were chopped up in 20% heat inactivated (56°C for 30 minutes) sera of rabbits in Fisher's medium No. 147G (Grand Island Biological Co., Grand Island, N. Y.). For the human tissues, 50% human serum was used. The cells were washed and resuspended in the same medium. To avoid bacterial contamination, the appendix was washed several times with Fischer's medium containing penicillin and streptomycin (100 units/ml and 100 μg/ml, respectively). The antibiotic mixture was also added to the final suspension of appendiceal lymphocytes.

The suspensions with and without prednisolone (10 μg/ml) were incubated at 37°C for 24 hours. The suspensions were also irradiated with X-rays (100 r, 100 Kv, 2 mm aluminum filter, 5 mA, one half value layer of 2.7 mm aluminum, the target distance-10 cm). Viable lymphocyte counts were made before and after incubation by methods described previously(3), and the number of viable lymphocytes per mm³ was calculated. The percentage survival of lymphocytes was based on the number of viable lymphocytes before incubation. The cytocidal effect produced by the reagent was calculated from the number of viable lymphocytes in suspensions incubated with and without reagent.

The phytohemagglutinin (PHA) used was PHA-P (Difco Laboratories, Detroit, Mich.) in doses of 0.3 and 1 μl/ml of suspension.

Results. 1. *Effect of prednisolone and X-rays.* Lymphocytes from the thymic glands of neonatal rabbits, rats and mice incubated in suspensions for 24 hours, showed good survival rates (Table I). There was no significant difference in survival of the cells among the 3 species. The survival rate of lymphocytes from human fetal thymus was relatively low (39.7%), possibly because of the prolonged time between excision of the tissue and preparation of suspension.

Prednisolone produced no significant cytocidal effect in 24 hours on thymocytes from newborn rabbits but killed practically all the thymocytes of newborn rat and mouse. It produced 77% cytocidal effect on neonatal human thymocytes.

The thymocytes of rabbits of various ages were also tested for sensitivity to prednisolone (Table II). There was a gradual increase in sensitivity and the thymocytes of 12-week-old rabbits had the same high degree of sensitivity as thymocytes of adult rabbits.

The spleen and appendix of newborn rabbits were too small to provide suitable lymphocyte suspensions. Lymphocytes from the spleen and the thymus of the 4-week-old rabbit were equally sensitive to prednisolone. However, the prednisolone-sensitivity of the splenic lymphocytes did not increase with

TABLE II. Effect of Prednisolone (10 μg/ml) and X-Irradiation (100 r) on Lymphocytes of Rabbit Thymus, Spleen, Appendix and Blood at Different Age Levels. Cells incubated for 24 hours at 37°C.

Source of lymphocytes	Age of rabbit	No. of exp	% Survival of untreated lymphocytes*	% Effect of prednisolone†	% Effect of X-rays†
Thymus	4 wk	9	75.2 ± 2.0‡	32.8 ± 3.5	43.0 ± 2.8
	8 "	5	67.7 ±13.7	69.6 ± 5.7	58.3 ± 6.8
	12 "	5	68.6 ± 2.2	82.8 ± 3.4	62.2 ± 4.5
	Adult	16	53.5 ± 3.1	88.4 ± 1.5	
Spleen	4 wk	11	37.1 ± 2.1	50.0 ± 3.4	57.3 ± 3.1
	8 "	5	41.7 ± 5.5	50.3 ± 8.8	72.5 ± 3.0
	Adult	19	50.4 ± 3.6	47.8 ± 2.7	60.1 ± 9.4
Appendix	4 wk	8	29.6 ± 2.7	63.4 ± 2.7	69.7 ± 4.1
	8 "	5	25.9 ± 3.8	54.1 ± 4.7	78.6 ± 3.4
	Adult	5	22.9 ± 2.5	48.7 ± 6.2	71.1 ± 4.3
Blood	Adult	5	73.4 ± 7.9	63.5 ± 2.2	71.4 ± 8.6

*, †, ‡ See footnotes of Table I.

age. The splenic, appendiceal and blood lymphocytes of the adult rabbit were significantly less sensitive to prednisolone than the thymic lymphocytes.

The thymocytes of newborn rabbits showed moderate sensitivity to X-irradiation in contrast to their resistance to prednisolone (Table I). The radiosensitivity of the lymphocytes from the thymus, spleen and appendix of rabbits did not show any significant variation with age (Table II).

2. *Cytology of viable cells and effect of PHA.* The human fetal thymic cells were round, 5-6 μ, with a few large round cells up to 10 μ in diameter. An occasional cell was in mitotic division during the first day of incubation. Incubation of the suspension with PHA for 1 to 5 days produced small clumps of 2 to 5 cells. Most of the cells were enlarged, round or elongated with small or moderate size nucleoli. These cells were classified as atypical lymphoblastoid cells since they differed from the classical lymphoblastoid cells that developed in PHA-treated blood lymphocytes of adults. The typical lymphoblastoid cell in the living state was usually larger, highly irregular in shape, and had many larger, irregular multiple nucleoli(4).

Incubation of neonatal and adult rabbit thymic cells with PHA (0.3 and 1 μl/ml) caused clumping, which interfered with viable cell counts. However, it appeared that fewer cells survived 1 to 3 days in the PHA-treated suspensions than in the controls. A few of the lymphocytes were enlarged and were diagnosed as lymphoblastoid cells. PHA caused the transformation of numerous human fetal thymic cells but of few, if any, rabbit thymic cells.

Discussion. A previous study has shown that lymphocytes from most sources were very sensitive to the cytocidal effect of prednisolone (1). For example, lymphocytes from the blood and spleen of rat and rabbit were nearly all killed when incubated with prednisolone for 1 or 2 days. In contrast, lymphocytes from some sources, such as blood and spleen of man and guinea pig, were relatively resistant to this reagent. The present study shows further pecularities in prednisolone-sensitivity of lymphocytes from different sources. Lymphocytes from the thymic glands of 2 human fetuses were more sensitive to the reagent than blood lymphocytes of adult men(2). In contrast, lymphocytes from the thymus of newborn rabbits were highly resistant. The differences in prednisolone sensitivity of lymphocytes from different sources cannot, as yet, be explained.

Lymphocytes from different sources also varied in their reaction to PHA. This reagent caused considerable clumping of thymic cells of neonatal and adult rabbit but produced few lymphoblastoid cells. On the other hand, PHA caused the enlargement and transformation of nearly all human fetal thymic cells into atypical lymphoblastoid cells.

There seems to be considerable variability

in the reported findings on the effect of PHA on thymic cells. However, the variability in findings can be eliminated to a considerable extent if we consider the age, species and the percentage of the thymic cells that react.

The present finding that PHA caused transformation of thymic cells of the human fetus is in agreement with observations of Bain and Gauld(5,6) and of Wilson(7). Thymic cells obtained from children and adults, on the other hand, did not respond to PHA according to several studies(8,9,10). In contrast, Claman(11) reported that 20% of the surviving thymic cells took up H^3-thymidine after 3 days of incubation with PHA. In comparison, about 70% human blood lymphocytes were transformed by PHA.

The discrepancy between the studies on post natal human thymic cells may be quantitative. All 3 studies are in apparent agreement that human thymic cells show a reduced capacity to react as compared to blood lymphocytes. Fuprthermore, Winklestein and Craddock(8) mention a slight increase in morphologically enlarged cells. It can be concluded that PHA caused a transformation of neonatal human thymic cells but produced little or no effect on thymic cells of children and adults.

Summary. Thymic cells from newborn rats and mice and from 2 human fetuses were killed by incubation with prednisolone (10 μg/ml) for 1 day. In contrast, thymic cells of newborn rabbits were resistant to the hormone. Thymic cells from young rabbits acquired increasing sensitivity with age and the cells from 12-week-old or older rabbits were nearly all killed by prednisolone. Lymphocytes from the spleen and appendix of rabbits were moderately sensitive to the cytocidal action of prednisolone. Many thymic cells from the human fetus were transformed by PHA into atypical lymphoblastoid cells but very few thymic cells from neonatal and adult rabbits were transformed.

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Growth Hormone-Releasing Activity in the Hypothalamus and Plasma of Rats Subjected to Stress.* (32228)

EUGENIO E. MÜLLER,† AKIRA ARIMURA, SHINJI SAWANO, TAKASHIGE SAITO AND ANDREW V. SCHALLY

Endocrine and Polypeptide Laboratories, Veterans Administration Hospital and Department of Medicine, Tulane University School of Medicine, New Orleans, La.

It has recently become clear that a variety of non-specific stimuli can induce alterations in growth hormone (GH) secretion in the rat(1,2). In addition to hypoglycemia(3,4), large doses of epinephrine, vasopressin, or exposure to cold result in a depletion of pituitary GH(1,2). By contrast, other types of stimuli, *i.e.*, injections of formalin and histamine, or laparotomy with exposure of the internal organs, induced only a slight but not significant depletion or rather elevation of

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† NIH International Post-Doctoral Fellow. Present address: Dept. of Pharmacology, University of Siena, Italy.