tem. Recently, Taylor *et al.*(12) have reported that the effect of carboline infusions upon aldosterone secretion in normal dogs was inconsistent. The failure of carboline to stimulate aldosterone secretion in simple hypophysectomized dogs, a preparation which responds to hemorrhage, salt depletion, inferior vena cava constriction, aortic constriction above the renal arteries, ACTH, renin and angiotensin with an increase in aldosterone secretion(2,3,4,5,6) casts doubt upon the role of this carboline in regulation of aldosterone secretion.

Summary. Injections of hog renin extracts with a purity of 24 units/mg protein and semipurified extracts of human kidney increased aldosterone secretion in hypophysectomized-nephrectomized dogs. Two erythropoietin preparations were inactive. Infusions of large doses of the angiotensin analogue (5-valine, 8-phenylalanine diamide) produced a minimal pressor response and only a slight increase in aldosterone secretion. Infusions of 1-methyl-6 methoxy-1, 2, 3, 4, tetrahydro-2-carboline into simple hypophysectomized or hypophysectomized-nephrectomized dogs did not stimulate aldosterone secretion.

The authors wish to thank Mr. Roy Shackelford, Mrs. Annette Lowe and Mr. Walter Hirniak for technical assistance. The angiotensin analogue was kindly supplied by Dr. Robert Schwyzer, Ciba Foundation, Basle, Switzerland, 1-methyl, 6-methoxy, 1, 2, 3, 4, tetrahydro-2 carboline by Dr. Gordon Farrell, hog renin by Drs. Weisenbaugh and S. W. Hoobler and erythropoietin preparations by Dr. D. C. Van Dyke.

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Received October 3, 1962. P.S.E.B.M., 1963, v112.

Interference by Newborn Lymphoid Cells with Establishment of Immunologic Unresponsiveness to a Protein Antigen.* (27934)

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Immunologic unresponsiveness to a protein antigen may be established at any time during the life of an animal, although with less facility as the animal matures. Smith *et al.*(1,2) quantitated the phenomenon in newborn and immature rabbits with bovine serum albumin and established a relationship between dosage and duration of the resulting unresponsiveness. While milligram amounts suffice in neonatal animals, others(3,4) have shown that several hundred times as much protein are required in adult animals.

Some investigators have suggested that the induction of tolerance during the neonatal period depends upon a certain immaturity of the lymphoid cells unique to this

* Supported by grant from U.S.P.H.S.

† Recipient of career development award.

TABLE I. Effect of Newborn Thymus Cells on Establishment of Immunologic Unresponsiveness in Newborn Mice.

Mouse strain	Dose of BSA at birth (mg)	Cell dose $\times 10^6$	Age at challenge (days)	Hemagglutination titers			
				<10	40-160	320-1280	>1280
A/Jax	none	none	30–40	—	1/19	4/19	14/19
"	10	"	30–40	12/14	2/14	—	—
"	10	5 –15* (isologous)	30–40	4/11	—	4/11	3/11
Swiss Webster	none	none	30	1/12	1/12	8/12	2/12
" "	10	"	30	11/13	2/13	—	—
" "	10	2.5–10*	30	—	—	4/6	2/6

* 2 of these received newborn spleen cells.

period of life and that the mechanism involved is therefore different from that observed in adult animals(5,6). However, it is possible that the relationship of the dose of antigen to amount or distribution of lymphoid tissue, or both, may be of definitive importance in this phenomenon(7).

The present study was undertaken in an attempt to determine whether differences between the lymphoid tissue of adult and newborn animals are qualitative or merely quantitative. Since lowering of the antigen dose at birth results in a shorter duration of unresponsiveness(1,7), increasing the number of lymphoid cells may have a similar effect. Investigations on the effect of changing the ratio of newborn lymphoid tissue to a tolerance-inducing dose of protein antigen are reported here.

Materials and methods. Newborn mice of the inbred A/Jax line‡ and of the random-bred Swiss-Webster strain were employed. Experimental and tolerance control animals were injected intraperitoneally within 24 hours of birth with 10 mg bovine serum albumin (BSA) (Armour Pharmaceutical Co., Kankakee, Ill.). Untreated control mice were also included in each litter.

Thymi and spleens were removed from newborn donors and cell suspensions prepared by teasing the organs with fine needles in tissue culture medium 199. The A/Jax mice all received isologous cells; the Swiss-Webster mice, homologous cells. An aliquot of each cell suspension was used for cell-counting. The cells were injected intraperitoneally from 2 to 6 hours after administration of the protein.

The animals received the first immunizing injection, 1 mg of alum precipitated BSA intraperitoneally, at the age of 30 to 40 days. A booster injection of 1 mg of BSA in soluble form was given 3 weeks later, and the animals were bled 1 week after the second injection.

Hemagglutination titers were determined according to the procedure described by Stavitsky(8). The tanned erythrocytes were sensitized with a purified BSA preparation obtained by starch block electrophoresis of the commercial material. The titers are expressed as the reciprocals of the highest dilutions giving agglutination.

Results. The data summarized in Table I show that, in both strains of mice, a neonatal injection of 10 mg BSA resulted in tolerance when the first immunizing injection was given 30 to 40 days later. Of such mice, 23 out of 27 showed no detectable antibody formation upon challenge; whereas, of the 31 control mice, 28 produced serum antibody titers >160, most of which were >1280. In the majority of animals (13 out of 17) given neonatal lymphoid cells shortly after the antigen injection, tolerance could not be demonstrated at the age of 30 to 40 days.

The small number of animals receiving spleen cells prevents any comparison between the effect of spleen and thymus cells.

Discussion. The results clearly show that lymphoid cells from newborn donors given 2 to 6 hours after a protein antigen injection

‡ The authors would like to thank Dr. E. A. Boyse, Dept. of Pathology, New York Univ. School of Med., for providing us with breeding pairs of A/Jax mice.

at birth can either prevent or abolish the state of immunologic unresponsiveness normally induced by the same dose of antigen alone.

The mechanism of this interference may be explained in several ways.

1. The injection of these lymphoid cells may merely serve to increase the number of immunologically competent cells early in the life of the recipient animal, and thereby change the ratio of lymphoid cells to antigen. If unresponsiveness is the result of reaching every immunologically competent cell with a critical concentration of antigen, it can be expected that, as the number of cells is increased, the dose of antigen required to affect every cell increases statistically.

2. The intraperitoneal injection of cells may result in a less effective contact between the reactive tissue and the paralyzing antigen than would normally be the case, *i.e.*, lymphoid cells become present in areas where they are normally lacking in a newborn animal.

3. The maturation of the cells from the lymphoid organs may have been affected by their dislocation from the supporting stroma. This last possibility has to be considered particularly in view of the fact that a difference has been reported(9) in the effectiveness of whole thymus and thymic cell suspensions in restoring the immunologic competence to thymectomized mice.

Further studies are under way to determine the relative effectiveness of thymus and spleen cells from newborn animals, and thymus cells from newborn and adult animals, in interfering with the establishment of immunologic unresponsiveness.

Summary. Injection of 5 to 15 $\times$ 10^6 newborn lymphoid cells into newborn mice, 2 to 6 hours following injection of a tolerance-inducing dose of BSA, resulted in immunologic responsiveness in 13 out of 17 mice upon challenge 30 to 40 days later. It is felt that quantity and distribution of lymphoid tissue are of major importance in determining the ease with which immunologic unresponsiveness can be established.

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Received October 17, 1962. P.S.E.B.M., 1963, v112.

Effect of Estrogen Administration *in vivo* on Prolactin Release by Rat Pituitary *in vitro*.*† (27935)

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In vitro cultures of anterior pituitary tissue for a number of days produce considerable quantities of prolactin(1). Such cultures are capable of synthesizing and releasing many times more prolactin than is found in fresh anterior pituitary tissue. Recently we reported(2) net prolactin synthesis by rat anterior pituitaries incubated in a Dubnoff shaker for only 2 hours. This short term incubation procedure has been found to be particularly appropriate to investigate problems concerned with control of prolactin release.

* Published with the approval of the Director of the Michigan Agri. Exp. Station as Journal Article No. 3069.

† This investigation was supported in part by re-grant from Nat. Inst. Health.