

TABLE II.
Enhancement of Bacterial Agglutination Titers by
Means of Serum Diluent.

Patient	Test antigen	Agglutinin titer	
		Saline diluent	Serum diluent
PN	Typhoid H	0	0
	" O	1:320	1:2,560
	Paratyphoid A	1:80	1:160
NL	" B	1:160	1:1,280
	Flexner V	1:80	1:640
	" Y	1:160	1:640
	Sonne	0	0
	Schmitz	1:80	1:640
JC	Shiga	0	0
EG	Flexner V	1:320	1:5,120
	<i>Brucella abortus</i>	1:40	1:160
	" <i>melitensis</i>	1:80	1:80
JB	" <i>suis</i>	1:40	1:80
	Typhoid O	0	0
	" H	1:320	1:1,280
CE	Paratyphoid A	0	0
	" B	1:160	1:1,280
	Paracolon	0	1:320

ophile agglutinins in both unabsorbed and absorbed serum were detected 5 days earlier with serum diluent. The confirmatory value of the guinea pig kidney absorption test in diagnosis of infectious mononucleosis⁹ is emphasized by the fact that serum diluent failed to enhance titers of absorbed serums in negative cases.

Bacterial Agglutination. Preliminary results

showing the enhancement of various bacterial agglutinin titers by means of serum diluent are given in Table II. It was noted that clumps of agglutinated bacteria in serum diluent were more easily dispersed on vigorous shaking than in saline. A similar enhancement of titer was observed with plasma, ascitic fluid, rabbit serum and 20% bovine albumin diluents. It is of interest that agglutinins for a paracolon bacillus isolated from a paratyphoid-like infection were demonstrable only by means of serum diluent.¹⁰

Comment. We feel that our results together with those previously reported by others suggest that not only does serum diluent inhibit the action of blocking antibody in certain serum, but that it is probably a more favorable medium in which the agglutination reaction can occur. Perhaps the presence of agglutinins can be detected sooner in the course of an infection by the routine use of serum diluent.

Summary. Heterophile and various bacterial agglutination titers were significantly enhanced by the use of serum diluent instead of saline. Similar results were obtained with plasma, ascitic fluid and bovine albumin diluents.

¹⁰ Milzer, A., unpublished studies.

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Effect of Estrogen on Plasma Vitamin A of Normal and Thyroidectomized Rabbits.*

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The congenital malformation in the eyes of young rats produced by a severe maternal deficiency of vitamin A during pregnancy,^{1,2}

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¹ Warkany, J., and Schraffenberger, E., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 49.

² Jackson, B., and Kinsey, V. E., *Am. J. Ophthalm.*, 1946, **29**, 1234.

appears to have a strong resemblance to the fibroplasia which has been observed to occur spontaneously behind the lenses of some premature human infants.³ Since it seems unlikely that this retrolental fibroplasia in humans is due directly to a maternal deprivation of vitamin A,² other influences which might cause

³ Terry, T. L., *Am. J. Ophthalm.*, 1942, **25**, 1409; *Arch. Ophthalm.*, 1943, **29**, 36, 54.

TABLE I.
Liver Vitamin A Level of Normal and Thyroidectomized Rabbits Treated with Large Doses of Estrogen.

	Thyroidectomized			Normal		
	No. of animals	Liver Vit. A I.U./g	Plasma Vit. A I.U. %	No. of animals	Liver Vit. A I.U./g	Plasma Vit. A I.U. %
Control	4	80	401	4	64	206
Estrogen treated	4	55	245	4	47	182

a general, or even a local, deprivation of vitamin A were sought.

During the course of pregnancy, the blood vitamin A level falls markedly and continuously so that an abnormally low level obtains at term. This low vitamin A level returns to approximately normal in about 24 hours after parturition.^{4,5} The behavior of the blood vitamin A during pregnancy appears to be the converse of that reported for the blood and urine estrogen levels; the estrogen output increases to a very high level and then drops precipitously directly after parturition.^{6,7} An earlier report has also suggested that estrogens may inhibit the growth-stimulating effect of carotene.⁸

It was considered to be of value, therefore, to establish whether a relationship exists between the administration of estrogens and the blood level of vitamin A. Since thyroid material has a marked influence on both of the above substances,^{9,10} the role of the thyroid has been taken into account in this study.

Experimental. Young animals were used in

order to reduce the effect of their own sex hormones to a minimum. Weanling, albino rabbits were maintained on a normal chow diet. The rabbits in Exp. I were all females from one litter and were thyroidectomized at 5½ weeks of age.

Two weeks after thyroidectomy, the intramuscular injection of estrogen (α estradiol benzoate in peanut oil[†]) was begun. An injection of 2,000 R.U. (0.33 mg) was given twice a day to each animal; one day a week only one dose was injected. Thus, 64,000 R.U. were given to each experimental animal over 17 days in Exp. I, and 68,000 R.U. over 19 days in Exp. II. The animals used in the second experiment were not thyroidectomized but were started on the estrogen treatment when approximately 6 weeks old. These rabbits were from 2 litters, 4 being females, and were distributed equally between the "control" and "estrogen treated" groups.

A blood sample was obtained by heart puncture before the first injection of estradiol and at various intervals during the course of the experiment. Finally, the animals were killed and the livers removed. The plasma samples were analyzed for their vitamin A content by the micromethod of Bessey, *et al.*¹¹ Aliquots of the livers were digested in 5-10 volumes of alcoholic KOH (5N KOH in 60% ethanol) on a hot water bath for 30 minutes and were then extracted with a 1:1 mixture of xylene and kerosene. The vitamin A concentration was then determined by the same procedure as that used for the determination in the plasma.

¹¹ Bessey, O. A., Lowry, O. H., Brock, M. J., and Lopez, J. A., *J. Biol. Chem.*, 1946, **166**, 177.

[†] Part of the estradiol benzoate used was kindly furnished by Dr. F. E. Houghton of the Ciba Pharmaceutical Products, Inc.

⁴ Jackson, B., and Kinsey, V. E., private communication.

⁵ Goldberger, M. A., and Frank, R. T., *Am. J. Obstet. Gynecol.*, 1942, **43**, 865.

⁶ Cole, H. H., and Saunders, F. J., *Endocrinology*, 1935, **19**, 199; Smith, G., Smith, O. W., and Pincus, G., *Am. J. Physiol.*, 1938, **121**, 98; Cohen, S. L., Marrian, G. F., and Watson, M. C., *Lancet*, 1935, **228**, 674.

⁷ Lund, C. J., and Kimble, M. S., *Am. J. Obstet. Gynecol.*, 1943, **46**, 486.

⁸ von Euler, H., and Klusmann, E., *Arkiv Kemi, Mineral Geol.*, 1932, **11B**, No. 1; *C. A.*, 1934, **26**, 3827.

⁹ McDonald, M. R., Riddle, O., and Smith, G. C., *Endocrinology*, 1945, **37**, 23.

¹⁰ Greaves, J. D., and Smith, C. L. A., *Am. J. Physiol.*, 1936, **116**, 456.

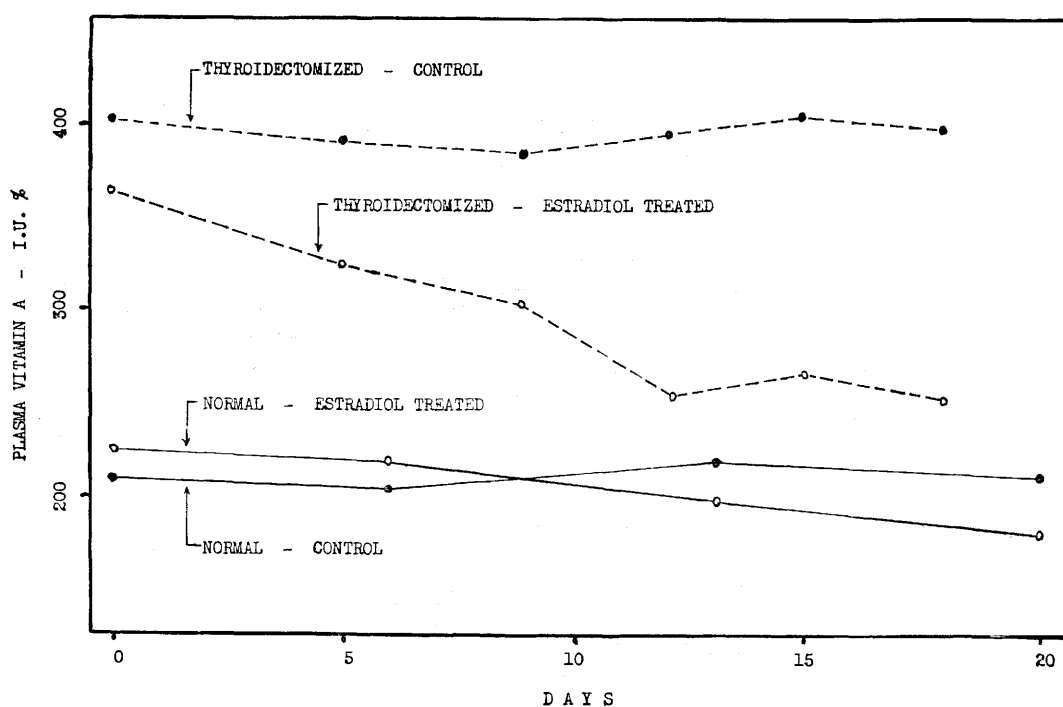


FIG. 1.

Plasma vitamin A as affected by the administration of α -estradiol benzoate. Broken line shows results obtained from thyroidectomized rabbits; solid line, from non-thyroidectomized normal animals.

The results of these experiments are shown in Fig. 1. The plasma vitamin A remained essentially unchanged in the control animals of both experiments. However, the level of vitamin A in the plasma of the estrogen treated rabbits showed a decline, particularly in the case of the thyroidectomized ones. The decrease observed in the non-thyroidectomized rabbits does not appear to be significant. It was found that the plasma level of vitamin A was higher in the thyroidectomized animals than in the normals. This higher level is probably due to the decreased metabolism of vitamin A observed after removal of the thyroid. The vitamin A content of the livers reflects the final plasma level determinations (Table I).

A possible explanation of the observed decrease in vitamin A may be found in the experiments of Loeb.¹² He reports that the

stores of body fat are increased under the influence of repeated estrogen injections. It would appear likely that the fat which is deposited may carry along some vitamin A, thereby lowering the plasma level.

The amount of estrogen used in these experiments was much greater than might be expected to be secreted by the pregnant animal. Yet, this large amount of estrogen did not depress the plasma vitamin A level below normal. It would then seem doubtful that the estrogens normally produced during pregnancy could influence the metabolism of vitamin A sufficiently to be considered a cause of the fibroplasia found in the eyes of premature infants.

Summary. Thyroidectomized and normal young rabbits were injected with large doses of estradiol benzoate for 17-19 days. The vitamin A level in the plasma of the thyroidectomized animals was significantly lowered. There was no appreciable decrease in the plasma vitamin A of the normal rabbits.

¹² Loeb, H. G., PROC. SOC. EXP. BIOL. MED., 1942, **51**, 330.