

positive for the methemoglobin-forming substance about one week before the blood culture became positive for pneumococci. There were also two cases of hemorrhagic colitis.

Summary. A substance capable of forming

methemoglobin from blood *in vitro*, and which can be quantitatively estimated as quinone, has been found to be excreted in certain pathological states. This urinary constituent interferes with the titration of ascorbic acid.

14577 P

Influence of Pituitary Adrenotrophic Hormone on Lymphoid Tissue Structure in Relation to Serum Proteins.*

ABRAHAM WHITE AND THOMAS F. DOUGHERTY.†

From the Departments of Physiological Chemistry and Anatomy, Yale University, New Haven, Conn.

Lymphoid tissue mass^{1,2} and blood lymphocytes³ are under pituitary adrenotrophic hormone influence. Loss in lymphoid tissue weight and the lymphopenia following hormone injection suggested an approach to the problem of the fate of lymphocytes.

This paper presents a preliminary description of lymphoid tissue histology after injections of adrenotrophic hormone into normal mice and rabbits. Histological alterations observed suggested study of effects of adrenotrophic hormone on serum proteins and antibody formation.

Histology. Single, subcutaneous injections of 1 mg adrenotrophic hormone⁴ in 0.5 ml of water into mice or 10 mg in 2 ml solution into rabbits produce alterations in lymphoid tissue histology. Within 1 to 3 hours, degenerative changes are evident in lymphocytes in

germinal centers of lymph nodes, in Malpighian follicles of the spleen, in the thymic cortex, and in Peyer's patches. Three to 6 hours after injection, depletion of lymphocytes in lymphoid tissue occurs, accompanied by marked edema. Degenerative changes are characterized by pycnosis and nuclear fragmentation in small or medium-sized lymphocytes. Phagocytosis of nuclear debris and red cells is accompanied by polymorphonuclear invasion of lymphoid tissues. Restoration of normal lymphoid structure begins after 9 hours and is still continuing 24 hours after hormone injection. These changes do not occur in adrenalectomized mice injected with adrenotrophic hormone.

The loss of lymphocytes from lymphoid tissue and from the circulation, and their absence from large capillary beds, suggested dissolution of these cells. In this event, significant quantities of protein nitrogen would be released for metabolic use. A portion of the protoplasmic nitrogen of the dissolved lymphocytes was sought in the serum proteins.

Serum Protein Observations. Total serum proteins and the specific gravity of whole blood were determined on tail vein blood by Kagan's methods.^{5,6} No significant alterations in whole blood specific gravity occurred. The serum protein data are presented in

* This investigation has been aided by grants from the Josiah Macy, Jr., Foundation, the International Cancer Research Foundation, and the Fluid Research Fund of Yale University School of Medicine.

† Fellow of the International Cancer Research Foundation.

¹ Dougherty, T. F., and White, A., *Proc. Soc. Exp. Biol. and Med.*, 1943, **53**, 132.

² Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., *Ibid.*, 1943, **54**, 135.

³ Dougherty, T. F., and White, A., *Science*, 1943, **98**, 367.

⁴ Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

⁵ Kagan, B. M., *J. Clin. Invest.*, 1938, **17**, 373.

⁶ Kagan, B. M., *J. Lab. and Clin. Med.*, 1941, **26**, 1681.

TABLE I.
Effect of Pituitary Adrenotrophic Hormone and of Adrenalectomy on Total Serum Proteins.

Animal	No. of animals	Treatment	Total serum proteins, g/%	P values†
Normal rat	22	None	6.00 ± 0.01*	
" "	8	3 hr after 3 mg A‡	6.22 ± 0.07	<0.01
" "	8	6 " " " " "	6.30 ± 0.08	<0.01
" "	4	24 " " " " "	5.81 ± 0.01	0.04
" mouse	19	None	6.02 ± 0.02	
" "	17	1 mg A daily, 15 days	6.52 ± 0.07	<0.01
Adrenalectomized mouse	16	0.025 mg D§ daily, 8 days	5.59 ± 0.08	<0.01

* Means and standard errors.

† P values, compared to controls.

‡ A = adrenotrophic hormone.

§ D = desoxycorticosterone acetate in oil (Schering).

Table I. After a single injection of adrenotrophic hormone, total serum proteins increase at the time lymphoid tissues undergo involution and lymphopenia occurs. Twenty-four hours after injection, the lymphoid tissue and blood lymphocyte count are almost normal,³ coinciding with approximately control serum protein values. Chronic hormone injections result in decreased lymphoid tissue weight,^{1,2} a trend toward persistent lymphopenia,³ and consistently elevated serum protein levels. Adrenalectomized mice, receiving desoxycorticosterone daily, show enlarged lymphoid tissues,⁷ a lymphocytosis,⁷ and subnormal serum protein concentration. In normal or adrenalectomized mice, the dose of desoxycorticosterone used produces no lymphopenia⁷ or lymphoid tissue involution.^{7,8} However, this dose is sufficient to prevent hemoconcentration in the adrenalectomized mouse. In the absence of desoxycorticosterone administration, hemoconcentration obscures the serum protein fall.

Comment. Injection of adrenotrophic hormone, or cortical extract, produces an absolute lymphopenia. A probable basis for the peripheral lymphopenia is the dissolution of lymphocytes in tissues following injection of either hormone. Since increased serum proteins occur simultaneously with washing out of lymphocytes as a result of adrenotrophic hormone action,[‡] lymphoid tissue is a possible

source of serum proteins.

The alterations observed in serum proteins (Table I) do not appear to be of great magnitude, although highly significant. However, when these changes are considered in the light of the circulation rate, and the period over which they persist, it is apparent that an increased quantity of protein is being added to the blood. A portion of this protein may undergo glyconeogenesis in the liver. Since the following communication⁹ establishes that adrenal cortical extract injection increases antibody titer, it is apparent that the globulins are one fraction of the serum proteins which were augmented in the present study.

Summary. Decrease of lymphocytes in lymphoid tissue and the concomitant lymphopenia following injection of pituitary adrenotrophic hormone, or adrenal cortical extract, are in their time relationships correlated with an increase in serum proteins. These same relationships persist in animals given daily injections of adrenotrophic hormone. Adrenalectomized mice, in which hemoconcentration is prevented, have a lower than normal concentration of serum proteins for at least 8 days following operation.

‡ The changes in lymphoid tissue structure, in blood lymphocytes and in serum proteins are essentially the same if adrenal cortical extract is used in place of adrenotrophic hormone in normal animals.

⁷ Unpublished results.

⁸ Wells, B. B., and Kendall, E. C., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 133.

⁹ Dougherty, T. F., White, A., and Chase, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 28.