(b) Testosterone stimulates the thyroid and parathyroid glands either by way of the anterior pituitary or through some other gland of internal secretion.

The second thesis seems more plausible, especially since the changes noted so closely parallel those in the ovary which we have shown are stimulated by way of the hypophysis. Experiments are under way at present to clarify these points.

Conclusion. Testosterone propionate is capable of stimulating the thyroid and parathyroid glands of the intact immature female rat. This stimulation is manifested by increased mitotic activity and by histological evidence of functional activity.

11320 P

The Mechanism of Action of Heparin*

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It is known that heparin does not prevent the coagulation of a fibrinogen solution by purified thrombin.[1] It likewise is unable to prevent the conversion of isolated prothrombin to thrombin.[2, 3] To exert its powerful anticoagulant action, heparin requires an additional factor which is known to be present in blood, plasma, and serum.[1, 4, 5] This factor has been shown by Quick[4] to be contained in the serum albumin fraction.

We have investigated the activity of various components of the albumin fraction in producing the anticoagulant effect of heparin on the fibrinogen-thrombin system.

The *thrombin* was obtained by the method of Eagle,[6] and the fibrinogen was prepared in the usual manner by repeated precipita-

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1 Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918-1919, **47**, 328.

2 Mellanby, J., *Proc. Roy. Soc. (London)*, 1934, B **116**, 1.

3 Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 391.

4 Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

5 Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

6 Eagle, H., *J. Gen. Physiol.*, 1935, **18**, 531.

tions with sodium chloride. *Heparin* was used in the form of the pure sodium salt.†

The activities of the albumin components studied are shown in Table I, which reproduces a typical experiment. The letters in the first column of this table stand for the following compounds: *A,* crystalline albumin from human serum, prepared by Dr. F. E. Kendall of the Research Division for Chronic Diseases, Welfare Island. We are greatly indebted to Dr. Kendall for different specimens of this substance. *B,* albumin from sheep plasma, prepared according to Howe.[7] *C,* fraction from human plasma insoluble at 55% saturation with ammonium sulfate, presumably containing the globoglycoid of Hewitt.[8] *D,* fraction from human plasma insoluble at 75% saturation with ammonium sulfate. *E,* fraction from human plasma insoluble at 100% saturation with ammonium sulfate. This fraction presumably contained the seroglycoid of Hewitt.[9] *F,* the supernatant solution after removal of E, dialyzed and concentrated by ultrafiltration.

All substances were dissolved in physiological saline and carefully adjusted to the neutral point. The pH measurements were performed by means of the glass electrode. The experiments were carried out at room temperature as follows: 0.1 cc of a thrombin solution (from human plasma), 0.06 cc of a 0.3% heparin solution, and 0.06 cc of the protein solution were mixed. Three minutes later

TABLE I.
Influence of Albumin Fractions on the Anticoagulant Effect of Heparin.

	30″	3′	5′	10′	15′	20′	25′
A, (9 mg N/cc) without heparin	+						
" " with "	+						
A, (4.5 mg N/cc) without heparin	+						
" " with "	+						
B, (1.4 mg N/cc) without heparin	—	—	+				
" " with "	—	—	—	—	—	—	—
C, (1.2 mg N/cc) without heparin	+						
" " with "	+						
D, (2.7 mg N/cc) without heparin	—	—	—	+			
" " with "	—	—	—	—	—	—	—
E, (1.2 mg N/cc) without heparin	+						
" " with "	—	—	—	—	—	—	—
F, (2.2 mg N/cc) without heparin	—	+					
" " with "	—	—	—	—	—	—	—

+ = clot.

† We wish to thank Hoffmann-LaRoche, Inc., Nutley, N. J., for the heparin preparation used.

[7] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

[8] Hewitt, L. F., *Biochem. J.*, 1938, **32**, 26.

[9] Hewitt, L. F., *Biochem. J.*, 1936, **30**, 2229; 1937, **31**, 360.

0.2 cc of a fibrinogen solution was added to the mixture. Fibrinogen preparations from human and sheep plasma gave essentially the same results. In the control experiments 0.06 cc of saline were substituted for the heparin. The tubes were examined for clots at fixed intervals in order to avoid unnecessary agitation. The concentration of the protein solutions used in the experiments reproduced in Table I is expressed in mg N per cc of protein solution.

As shown in Table I, the crystalline albumin fraction even when tested in fresh solution is entirely inactive as a complement of heparin. The activity appears to reside in the most soluble fraction of the serum albumin. It might be mentioned that the albumin solutions prepared by Howe's method retained their activity for more than 50 days. It is not possible to state definitely whether the activity is due to a single component of the albumin fraction. Additional work will have to be carried out with respect to this question. A detailed report on this work and related aspects will be published at a later date.

It might be mentioned that occasionally individuals are encountered whose clotting time responds to heparin to a slight degree only. It will be of interest to see whether the heparin complement here discussed is lacking in the serum of these patients.

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Chloride Excretion in Hypothyroidism.

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Cutler, Power and Wilder,[1] using a standardized sodium chloride depletion test, have shown that the chloride concentration in the urine of patients with Addison's disease is significantly and consistently higher than in controls. These observations have been confirmed by others.[2] Six of 8 patients with clinical hypopituitarism, studied under similar conditions, have shown increased concentrations of chloride in the urine comparable to those found in patients with

1 Cutler, H. H., Power, M. H., and Wilder, R. M., *J. Am. Med. Assn.*, 1938, **111**, 117.

2 Dryerre, H. W., *Edinburgh Med. J.*, 1939, **46**, 267.