human prostate phosphatase. Both are inhibited by sodium fluoride and by propyl alcohol, neither is accelerated by Mg ion: an extract of rat preputial gland with 102.5 units of "acid" phosphatase activity showed 65.0 units with *M* propyl alcohol; 7.0 units with *M*/200 NaF, and 99.0 units with *M*/50 $MgCl_2$. Rat preputial glands, like rat prostate glands, also contain significant amounts of "alkaline" phosphatase (11.6 to 50.1 units per g fresh tissue at pH 9.0).

These results suggest another approach to problems of the accessory sex glands. It is interesting to note in this connection that Disselhorst long ago,[10] on purely morphologic grounds, postulated a specific function of the rat preputial glands.

Methods. The ground glands were usually extracted with 10 times their weight of distilled water for 24-48 hours in the refrigerator. Further dilution of the extract as high at 1:500 was necessary for optimal hydrolysis in the cases of human prostate and rat preputial glands. Phosphatase activity at pH 4.9 was determined by the adaptation of the King and Armstrong method[11] described elsewhere.[7] The unit employed in this study is defined as that degree of phosphatase activity which at pH 4.9 and 37°C will liberate from the specified buffer-monophenylphosphate substrate solution 1 mg of phenol in 1 hour.

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Effect of Calcium in Quantitative Determination of Prothrombin.*

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Recent investigations,[1, 2] indicate that satisfactory methods for the quantitative determination of prothrombin have been developed.

10 Disselhorst, R., in Oppel's *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere*, 1904, **4**, 280.

11 King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

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1 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

These methods are dependent on the original suggestion of Quick, Stanley-Brown and Bancroft[3] that the coagulation time of oxalated plasma, when mixed with an excess of thromboplastin and then recalcified is a direct measure of the prothrombin content of the plasma. Subsequent studies have led some investigators to conclude that the defect in the coagulation of the blood in certain cases of jaundice is due to a deficiency of prothrombin.

The methods followed in the present studies were essentially the same as those described by Quick.[1, 4] The prothrombin time was determined on 0.1 cc portions of oxalated plasma in clean, dry 100 x 13 mm test tubes in a water bath at 37.5°C. An excess of thromboplastin was supplied by the addition of 0.1 cc of a freshly prepared saline suspension of an acetone extract of rabbit brain. Theoretically the thromboplastic preparation served to convert all of the prothrombin to thrombin. Upon recalcification with 0.1 cc of a 0.025 M calcium chloride solution, which was considered to be the optimal amount of calcium,[1] it was observed that the results were extremely variable. Furthermore, it was usually impossible to obtain a fibrin clot in normal plasma in from 12 to 13 seconds[1] unless the concentration of calcium was altered.

Eleven solutions of calcium chloride varying in concentration from 0.1 M to 0.000625 M were prepared. The effect of each of these solutions as a recalcifying agent in the determination of prothrombin was studied on plasma obtained from 20 normal individuals. In each instance the results were entirely similar. The observations in one typical case are shown in Table I. The data

TABLE I.
Effect of Calcium Concentration on Prothrombin Time of Normal and Pathological Subject.

Molar conc. of CaCl	Coagulation time in seconds	
	Normal	Jaundice
.1	48	340
.05	24	73
.033	19	49½
.025	15½	40
.02	13½	38
.015	13	32
.01	12	32
.005	11	29
.0025	10	29
.00125	15	50
.000625	38	124

[3] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sc.*, 1935, **190**, 501.

[4] Quick, A. J., personal communication.

obtained on one of 4 cases of obstructive jaundice with a hemorrhagic diathesis are presented in the table for comparison The results clearly show that an excess of calcium as well as an insufficient amount of calcium prolonged the time required for the coagulation of normal and pathological plasma. The optimal amount of calcium required varied among normal individuals. Usually recalcification with 0.1 cc of a 0.0025 M calcium chloride solution resulted in a minimal coagulation time of 10 seconds. However, in certain instances a minimal coagulation time occurred when recalcification was carried out with 0.01, 0.005, and 0.00125 M calcium chloride solutions.

It is well known that an excess of any neutral salt preserves the fluidity of the blood. Horne,[5] Sabbatini,[6] Rettger,[7] and Stassano and Daumas[8] observed the anticoagulant properties of large amounts of calcium salts.

If the quantitative determination of prothrombin is to be reliable there must be no variables except the prothrombin. The present studies indicate that calcium is a variable and that in order to obtain a minimal coagulation time (true prothrombin time) the optimal amount of calcium necessary for recalcification must be determined in each instance.

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Use of Cyanide in the Determination of Ascorbic Acid.

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Though blood plasma or serum inhibits the catalytic oxidation of ascorbic acid as shown by De Caro and Giani[1] and Mawson,[2] Barron, Barron, and Klemperer[3] found that this protective property of plasma did not completely prevent such oxidation. Kellie and

5 Horne, R. M., *J. Physiol.*, 1896, **19**, 356.

6 Sabbatini, L., Abstr. in *Mosso's Arch. ital. de biol.*, 1901, **36**, 416.

7 Rettger, L. J., *Am. J. Physiol.*, 1909, **24**, 406.

8 Stassano, H., and Daumas, A., *Compt. rend. Acad. Sc.*, 1924, **150**, 937.

1 De Caro, L., and Giani, M., *Z. f. physiol. Chem.*, 1934, **228**, 13.

2 Mawson, C. A., *Biochem. J.*, 1935, **29**, 569.

3 Barron, E. S. G., Barron. A. G., Klemperer, F., *J. Biol. Chem.*, 1936, **116**, 563.