

of the tubular transfer of xylose¹⁴ would indicate that the differences in the tubular absorption for glucose and galactose is not to be attributed to the absence of an active mechanism for the transfer of galactose. The tubular transfer of galactose and xylose is quite similar in several respects; the fraction of the filtered sugar that is absorbed is independent, in both cases, of the concentration of the sugar in the plasma, and of moderate changes in the degree of concentration of the glomerular filtrate. A demonstrated difference lies in the magnitude of the fraction absorbed. In contrast to 40% for galactose only 27% of the filtered xylose is absorbed. The observation that elevation of the plasma glucose level completely and reversibly blocks the tubular absorption of xylose, together with conclusions drawn from a sound, critical analysis of the kinetics of transfer mechanisms, has led Shannon¹⁴ to conclude that xylose is absorbed by an active tubular process which is identical

¹⁴ Shannon, J. A., *Am. J. Physiol.*, 1938, **122**, 755.

with that responsible for the absorption of glucose. Studies involving the use of phlorhizin would appear to support such a conclusion. Since galactose is absorbed by the renal tubules at a greater rate than xylose, it seems reasonable to conclude that the tubular absorption of the galactose also involves an active transfer mechanism. The difference in behavior of glucose and galactose, including the differences in their response to thyroxin, may then be attributed to a difference in the rate determining step in the sequence of reactions responsible for the active transfer.

Summary. The renal tubules of the dog absorb about 40% of the galactose that appears in the glomerular filtrate; within rather wide limits the fraction that is absorbed is independent of the concentration of plasma galactose and of the degree of concentration of the glomerular filtrate. In contrast to glucose, the renal tubular transfer of galactose is not altered by the administration of thyroxin. It is likely that a transfer mechanism is involved in the tubular absorption of this sugar.

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Electrophoresis of Purified Prothrombin.

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Prothrombin has not been identified as a separate component in the electrophoresis patterns of oxalated or citrated plasma, because it is not present in sufficient concentration to permit detection. For this reason extensive electrophoretic data can be obtained only on concentrated preparations of prothrombin. In previous purification work^{1,2} impurities have been removed in progressively greater proportion. In recent work we have obtained preparations of still higher purity.

This offered an opportunity to study the electrophoretic properties of prothrombin for the first time.

The Tiselius apparatus used for this work is equipped with a Schlieren type lens and a Svensson diaphragm. The mobilities were calculated from observations of the descending boundary, in accordance with the recommendations of Longworth and MacInnes.³ The values for any given pH represent the average of several determinations. A new prothrombin preparation was made for each run. It is planned to offer a comprehensive presentation

¹ Seegers, W. H., Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Biol. Chem.*, 1938, **123**, 751.

² Seegers, W. H., *J. Biol. Chem.*, 1940, **136**, 103.

³ Longworth, L. G., and MacInnes, D. A., *J. Am. Chem. Soc.*, 1940, **62**, 705.

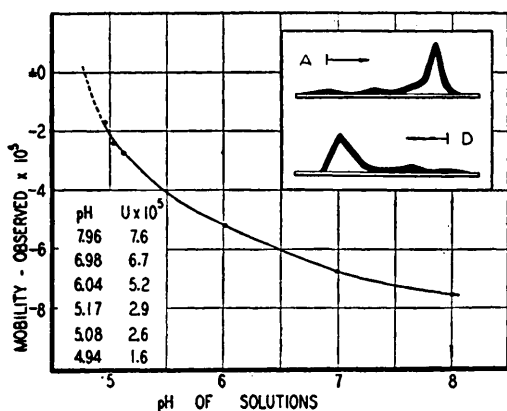


FIG. 1.

of the purification procedures at a later date.

A typical pattern, reproduced in Fig. 1, shows that the prothrombin component represents 80 to 90% of the total proteins in this preparation. The latter possessed 12,000 units⁴ of prothrombin per mg tyrosine. By calculation pure prothrombin can be expected to have from 13,000 to 15,000 units activity per mg tyrosine or roughly 1,300 to 1,500 units per mg dry weight.

With similar preparations the mobility was studied by varying the pH. The curve obtained is given in Fig. 1. These results were obtained with numerous different preparations, dissolved in veronal, acetate, or phosphate buffers of 0.1 ionic strength. In the region of pH 6, 7, and 8 the mobility of prothrombin is greater than that reported or obtained by ourselves for the more concentrated plasma protein constituents such as albumin, fibrinogen, or the α , β , and γ globulins.

⁴ Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

Since prothrombin is denatured in the region of or below pH 4.9, it was impossible to determine the isoelectric point accurately. By extrapolation it seems likely that it is pH 4.8 or less. It is obviously not pH 5.3 as might have been expected from the fact that prothrombin can be precipitated from diluted bovine plasma at pH 5.3. It is of interest to recall that acids inactivate prothrombin at pH 4.8.² Consequently it is almost certain that the isoelectric point and inactivation point are identical.

It has been shown that the conversion of prothrombin to thrombin can be blocked by heparin and an unidentified plasma factor.⁵ Moreover, heparin alters the electrophoretic properties of certain plasma constituents.⁶ It seemed desirable, therefore, to determine whether heparin alters the electrophoretic properties of prothrombin. The addition of heparin, in 0.4% concentration, produced no change in the mobility at pH 7.0.

Summary. The electrophoretic mobility of bovine prothrombin is greater than that of the more concentrated plasma protein constituents. At pH 7, heparin does not alter the mobility of prothrombin. The isoelectric point of prothrombin is in the region of pH 4.8 and in all probability is identical with the inactivation point. It is estimated that pure prothrombin will possess 1,300 to 1,500 units activity per mg of protein.

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

⁶ Chargaff, E., Ziff, M., and Moore, D. H., *J. Biol. Chem.*, 1941, **139**, 383.