tives are preferentially absorbed and retained in the proximal tubule cells, the exclusive site of active glucose reabsorption.[2, 9] He concluded that the concentration of phlorizin at its point of action within the kidney cells might be several times as great as m/1000.

The observations of Kalckar and the data presented here demonstrate that the hexokinase of rabbit kidney cortex is far more sensitive to phlorizin poisoning than the hexokinases studied by Lundsgaard. These various considerations indicate that (X), the minimum concentration of phlorizin required to completely inhibit glucose reabsorption *in vivo,* and (Y), the minimum concentration required to completely inhibit glucose phosphorylation *in vitro,* are close enough together to satisfy Lundsgaard's phosphorylation hypothesis of glucose reabsorption.

Although the phosphatase activity of rat kidney cortex extracts is quite sensitive to phlorizin at pH about 5, it shows practically no sensitivity in the intracellular pH zone (about 6.9).[10] It therefore seems unlikely that interference with glucose reabsorption by phlorizin can be due to any appreciable extent to inhibition of dephosphorylation.

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Effect of Vitamin D on Prothrombin Deficiency in the Rat.

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It has been reported[1] that the prothrombin deficiency produced in rats by a diet containing 20% mineral oil was corrected by vitamin K, and definitely improved by vitamin D administration. Previously, Smith and coworkers[2] had reported that vitamin D failed to improve the prothrombin deficiency in a biliary fistula dog, and Greaves[3] stated that massive doses of D subcutaneously were without benefit in biliary fistula rats. However, vitamin D has been shown to

9 Walker, A. M., Bott, P. A., Oliver, J., and MacDowell, M. C., *Am. J. Physiol.,* 1941, **133**, 480.

10 Chambers, R., *Bull. Nat. Research Council,* No. 69, 1929, Washington, D.C.

1 Eliot, Isaacs, and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 240.

2 Smith, Warner, Brinkhous, and Seegers, *J. Exp. Med.,* 1938, **67**, 911.

3 Greaves, *Am. J. Physiol.,* 1939, **125**, 423.

be of value in treating the bleeding tendency in clinical jaundice.[4] In view of these conflicting reports, we have further investigated the effect of vitamin D on prothrombin deficiency in the rat.

A prothrombin deficiency was produced in 36 normal rats by means of the diet described by Eliot, Isaacs and Ivy[1] (Table I, Group 1). Quick's method[5] for the estimation of prothrombin was used. After 29-52 days on the diet, the prothrombin time was prolonged from an average normal of 17.6 seconds to a mean of 59.7 seconds.

Eighteen rats whose prothrombin times had been increased by the diet from a normal average of 18.0 seconds to 53.4 seconds were given 500 units of vitamin D* subcutaneously. One week later the average prothrombin time of the group was 55.4 seconds (Table I, Group 2). There is no significant difference between the average values before and after treatment with vitamin D. Individually, 5 of the 18 appeared significantly improved. Ten of these rats were then given an additional dose of 1000 units of vitamin D subcutaneously (Table I, Group 3). One week later their average prothrombin time was 71.8 seconds compared to 41.2 seconds before the second dose was given. Again in this group, vitamin D failed to improve the prothrombin deficiency.

TABLE I.

		Prothrombin time—seconds															
								After Vitamin D									
								1 week (500 U)			3-5 days (1000 U)			1 week (1000 U)		
Group No.	No. rats	Controls			After diet												
		M	R	σ	M	R	σ	M	R	σ	M	R	σ	M	R	σ
1	36	17.6	13.6 22.4	.017	59.7	18.8 178.1	5.5									
2	18	18.0	14.9 22.0	.46	53.4	34.4 105.1	4.7	55.4	26.9 103.3	5.4						
3	10	17.7	16.1 22.2	.62	48.3	35.9 82.4		41.2	27.7 60.5	4.13				71.8	31.2 156.6	14.6
4	15	18.0	14.7 19.5	.37	71.6	39.3 178.1	9.9				50.8	18.3 **98.8**	6.5	50.2	29.6 **101.0**	5.6

M = Mean.
R = Range.
σ = Standard Error of Mean.

[4] Gray and Ivy, *Am. J. Dig. Dis.*, 1935, **2**, 368; McNealy, Shapiro, and Melnick, *Surg., Gynec., and Obst.*, 1935, **60**, 785.

[5] Quick, *J. A. M. A.*, 1938, **110**, 1659.

* Abbott Viosterol.

Since these results disagreed with those previously reported,[1] a third group of 15 deficient animals was given 1000 units of D† subcutaneously and the prothrombin time was determined 3-5 days and 1 week later (Table I, Group 4). In this group, the mean prothrombin values before and after treatment show a questionably significant difference. If the extreme variates are omitted in calculating the mean in column 6, the differences become insignificant. Individually, 7 of the 15 were definitely improved, 4 were more deficient after receiving the vitamin than before, 3 were essentially unchanged, and 1 died before a second determination was made.

Comment. These results fail to show that vitamin D has any definitely beneficial effect on the prothrombin deficiency produced by dietary means in the rat. Although certain individual cases showed improvement, the average results of the group show no striking effect. This does not agree with the interpretation of results previously reported.[1] The reason for this difference is not immediately apparent; however, the range of variation in the deficient rats is so great that it is difficult to evaluate any procedure unless a large series is used. This may explain the difference. The present results agree with those of Smith and coworkers[2] and of Greaves.[3] However, they fail to explain the beneficial effects of viosterol in clinical jaundice.

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Use of Avidin in Studies on Biotin Requirement of Microorganisms.*

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The recent discovery of anti-vitamins suggests a new principle in nutritional pathology, with far-reaching applications in the study of bacterial metabolism. Avidin,[1, 2] the first anti-vitamin to be iso-

† Meade-Johnson Special Concentrate.

* Presented at the forty-third general meeting of the Society of American Bacteriologists, Baltimore, Maryland, December 29, 1941.

1 Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1940, **136**, 801.

2 Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1941, **140**, 535.