chloride or sodium acid phosphate simultaneously with the sulfanilamide. These experiments indeed showed a reduced rate of conjugation in the blood and a somewhat decreased rate of elimination of the drug as compared with controls, as shown in Part 4 of Table I. The effects, however, were not as pronounced as in fasting. The lower peak in the blood under these conditions as compared with controls suggests a reduction in the rate of absorption of the drug. Obviously more work is needed to elucidate the effects of fasting or low protein diet on the fate of sulfanilamide in the body.

Summary. Prolonged fasting in the rabbit favors the absorption and retention of sulfanilamide, producing higher blood levels of the drug and over a longer period of time as compared to normally fed animals. This also favors reduction of acetylation of the drug. An acid producing diet or the administration of drugs favoring a state of acidosis appears to have an effect on the fate of sulfanilamide similar to that of fasting.

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Action of Phlorizin on Acid Phosphatase Activity and on Glucose Phosphorylation of Kidney Cortex Extracts.*

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1. *Phosphatase Experiments.* Several authors[1, 2, 3] have reported that phlorizin does not appreciably affect the phosphatase activity of kidney extracts. In all cases their findings are apparently concerned with the alkaline phosphatase, since the determinations were made within the pH range of 7.6 to 9.2. It was thought of interest to determine the effects of phlorizin on the acid phosphatase activities of kidney cortex and intestinal mucosa extracts.

The filtered extracts were prepared as described by Kay,[4] brought

* The author is indebted to Dr. C. H. Fiske for animal adenylic acid, and to Miss Ethel Shiels and Dr. Marshall Smith for many glass electrode pH determinations.

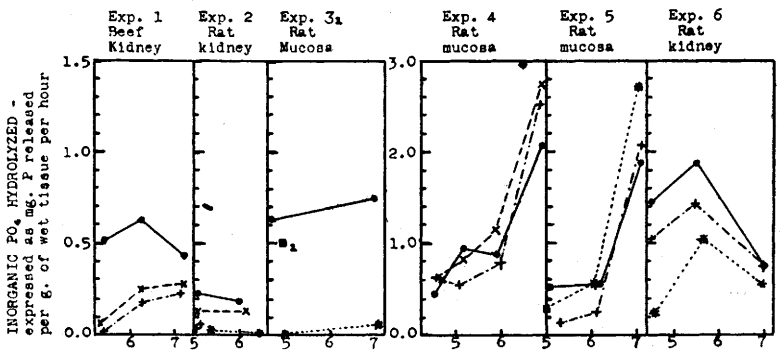
1 Lambrechts, A., *Arch. intern. physiol.*, 1937, **44** Suppl., 136.

2 Walker, A. M., and Hudson, C. L., *Am. J. Physiol.*, 1937, **118**, 130.

3 Kritzler, R. A., and Gutman, A. B., *Am. J. Physiol.*, 1941, **134**, 94.

4 Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

FIGURE I - PHOSPHATASE ACTIVITIES IN ACID pH RANGE



pH Values of the Hydrolysis Mixtures.

●———● Control hydrolysis mixtures—no phlorizin.

×— — —× Hydrolysis mixtures containing M/1000 phlorizin.

+ — · — — + Hydrolysis mixtures containing M/300 phlorizin.

* · · · · · * hydrolysis mixtures containing M/100 phlorizin.

[1]In all experiments except No. 3 glycerophosphate was used as substrate; in this experiment hexosediphosphate served as substrate. The square symbol in Exp. 7 represents a control value obtained using glycerophosphate.

to pH 5.2 by addition of 1 part in 7 of veronal-acetate buffer, to preserve the acid phosphatase, and kept on ice until used. Determinations of phosphatase activity were carried out at 37°C as described by Belfanti and coworkers.[5] The results are shown in Fig. 1.

At pH about 5 the rate at which inorganic phosphate is formed from glycerophosphate in the presence of kidney cortex extracts is markedly decreased by m/100 phlorizin, and in some instances is appreciably decreased by m/300 and even m/1000 phlorizin. With increase in pH the inhibition becomes less, until at and beyond pH 7 (up to pH 9) even m/100 phlorizin has practically no inhibitory action on the phosphatase activity.

When intestinal mucosa extracts were employed marked inhibition was secured only in Exp. 3, in which hexosediphosphate was employed as substrate. In 2 other experiments, using glycerophosphate as substrate, the results at pH below 7 were irregular, and at pH about 7 phlorizin actually produced an acceleration of the rate of disappearance of inorganic phosphate. In the alkaline pH range (7 to 9) the values for control tubes and for phlorizin-containing tubes were practically identical.

II. *Glucose Phosphorylation Experiments.* Kalckar[6] has succeeded in demonstrating phosphorylation of glucose in the presence

[5] Belfanti, S., Contardi, A., and Ereoli, A., *Biochem. J.*, 1935, **29**, 517.

[6] Kalckar, H., *Enzymologia*, 1937, **2**, 47.

of rabbit kidney cortex extracts. He has reported that this phosphorylation is strongly inhibited by phlorizin (about 60% by m/600) and has revived Lundsgaard's hypothesis[7] that phlorizin prevents glucose reabsorption by inhibiting glucose phosphorylation within these cells. In view of this hypothesis and Kalckar's observations it was thought of interest to determine the lowest concentration of phlorizin capable of producing practically complete inhibition of glucose phosphorylation by kidney cortex extracts, since we have estimates[1, 8] of the lowest *in vivo* concentration of phlorizin required to completely inhibit glucose reabsorption by the kidney (see below). Experiments similar to those of Kalckar have therefore been performed.

Ground up rabbit kidney cortex-sand brei was mixed with m/15 Na_2HPO_4 (1.5 cc for each gram of tissue), vigorously shaken for 10 minutes, and the clear supernatant fluid obtained on centrifuging used for the experiment. Two arm tubes were used. 1.0 cc of enzyme extract was placed in one arm, 0.5 cc of fluid containing other substances in the other. After temperature equilibration in a water bath at room temperature (about 22°C), the contents of the two arms were mixed, the time noted, and an aliquot blown into 10% trichloracetic acid for determination of original inorganic phosphate. At desired intervals other aliquots were taken. About the middle of the 1- or 2-hour experimental period a glass electrode pH determination was made on the phosphorylation mixture. Throughout the experimental period the tubes were rocked back and forth in the water bath to assure aeration of the mixture.

NaF was used in n/30 final concentration. When present the concentration of adenylic acid in the phosphorylation mixture was 5 mg %, that of sodium succinate n/150 and that of hexose 1.5%. By analysis that of inorganic phosphate was about 50 mg %.

The results are shown in Table I. The lowest concentration of phlorizin which will still produce practically complete inhibition of glucose phosphorylation is about m/333. It is interesting to note that very low concentrations of phlorizin (m/3333 and m/10,000) increase the rate of disappearance of inorganic phosphate.

Discussion. Lundsgaard[7] found that the minimum concentration of phlorizin required to markedly inhibit hexose phosphorylation brought about by muscle brei and dried yeast is about m/50 to m/100, and suggested that phlorizin may inhibit glucose reabsorption in the kidney by interfering with its phosphorylation. Later[8] he

[7] Lundsgaard, E., *Biochem. Z.*, 1933, **264**, 209.

[8] Lundsgaard, E., *Skand. Arch. f. Physiol.*, 1935, **72**, 265.

TABLE I.

Mg of Inorganic Phosphate P Disappearing, per Gram of Wet Kidney Cortex Tissue per Hour, in Presence and Absence of Adenylic Acid, Glucose, and Phlorizin.

Exp. No.	A.A.* present?	Sugar used	Conc. of phlorizin	pH of exper. mixture	Inorg. P disappearing: As mg	Inorg. P disappearing: As % of avg control value
I	Yes	0	0	—	0.01	1
		Glucose	0	7.17	1.46 Avg.	
				7.23	1.14 1.18	100
				7.17	0.93	
			m/333	7.29	—0.02	— 2
			m/1000	7.39	0.00	0
II	Yes	Glucose	0	6.80	1.12 Avg,	
				7.20	1.17 1.34	100
				7.30	1.62	
			m/500	7.25	0.40	30
			m/1000	7.12	1.69	126
			m/3333	7.22	1.71	128
			m/10,000	7.30	1.91	147
III	No	0	0	7.20	0.34	35
	Yes	0	0	7.41	0.55	57
		Glucose	0	7.31	0.88 Avg,	
				7.50	1.06 0.97	100
			m/333	7.42	0.34	35
			m/1000	7.34	0.90	93
			m/3333	7.32	1.10	113
			m/10,000	7.21	1.21	125
IV	No	0	0	6.97	0.10	
	Yes	0	0	7.31	0.33	
	Yes	Glucose	0	6.98	0.96	100
			m/333	7.08	0.02	2
		Fructose	0	6.89	1.89	100
			m/333	—	0.05	3
		Galactose	0	7.14	0.72	100
			m/333	7.20	—0.10	— 14

*A.A. equals 5 mg% adenylic acid plus n/150 Na succinate present or not present in the phosphorylation mixture.

estimated that the minimum *in vivo* concentration of phlorizin required to practically completely inhibit glucose reabsorption by the dog kidney is of the order of 0.5-1.0 mg per g of kidney tissue (0.75 mg = m/630), and concluded that the phosphorylation hypothesis was not entirely satisfactory. By another method, Lambrechts[1] arrived at a value of m/1000 phlorizin or less, as compared with Lundsgaard's value of about m/630. However, Lambrechts also found that 2 highly colored and strongly glycosuric phlorizin deriva-

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.