ditions L-, DL-, and D-aspartic acids had nearly equal activities. Methods for the separate microbiological determination of L-aspartic acid and total (L- and D-) aspartic acid with *Leuconostoc mesenteroides* were suggested.

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Effect of Phlorhizin on Intestinal Absorption of Glucose, Galactose, Fructose, Mannose, and Sorbose. (18106)

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Although phlorhizin has repeatedly been shown to inhibit the intestinal absorption of glucose(1-8), the extent of inhibition reported has varied from 30%(7) to 90%(1). The effect of this glucoside on the absorption of hexoses other than glucose has not been investigated as thoroughly. Wertheimer(6), using the Cori technic, measured the absorption of glucose, galactose, fructose, and mannose by pairs of unanesthetized rats. One rat of each pair received an alkaline solution of 100 mg of phlorhizin in sugar solution by gastric tube; the control received only the sugar solution. Although he used only 2 pairs of rats each to examine fructose and galactose, and only one pair for mannose, he concluded that the inhibition exerted by phlorhizin upon glucose absorpton applied also to the absorption of galactose, fructose, and mannose. Yoshikawa(8), using 2 consecutive 30 cm segments of rabbit jejunum, introduced 5.4% solutions of various sugars into these loops and removed samples for analysis at regular intervals. By putting into one of the loops a solution of phlorhizin, sugar, and sodium bicarbonate, and into the other loop only the sugar and bicarbonate, he was able to use the same intestine both for control and experiment. He found that the order of absorption for the sugars tested was: galactose, glucose, mannose, arabinose, xylose, and fructose. The greatest phlorhizin inhibition appeared to be upon galactose absorption, the least upon fructose.

Cori(9) and Wilbrandt and Laszt(10) have found glucose and galactose to be rapidly absorbed, mannose and the pentoses to be absorbed quite slowly, and the absorption rate of fructose to be intermediate between that for glucose and that for mannose. Wertheimer's(6) data do not include the various rates of absorption, since he measured the percentage of sugar absorbed. Yoshikawa's (8) finding that fructose was absorbed more slowly than mannose, or even xylose, suggested the need for further experiments along this line.

Experimental. The experiments were performed on albino and hooded rats of both sexes selected from stock raised in this laboratory. Absorption was studied by means of a technic employing the entire small intestine. Prior to the experiment the rats received 6 subcutaneous injections of a 10% suspension

* This investigation was performed in partial fulfillment of the requirements for the degree of Master of Science in the Department of Physiology, State University of Iowa.

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of phlorhizin in sesame oil (each dose being 0.25 cc/100 g) at intervals of 12 hours. The control animals, which were paired with the experimentals for sex and strain, received similar doses of the oil alone. A few rats were not injected with oil; since this group did not differ from the oil-injected controls, the data on both groups were pooled as controls. For 2 days before the experiment the rats received no solid food in order to clear the intestinal tract of reducing material, but were given a low residue meal of 3.3 cc of evaporated milk/100 g body weight, by gastric tube, one day preceding the experiment. The rats also received water by stomach tube at 12 hour intervals during the fast in order to further promote removal of food and debris from the intestine. At the end of this time a sample of urine was tested with Benedict's qualitative reagent(11) and showed in every phlorhizinized animal a copious glycosuria, while the control urines were always negative for sugar.

Each rat was then anesthetized with an intraperitoneal injection of Nembutal (45 mg/kg), the abdomen opened, and ligatures placed at the pyloric and caecal ends of the small intestine. The rats were kept warm (rectal temperature about 38°C) during the procedure. A measured volume (according to the animal's weight) of a 5.5% solution of the hexose being studied was injected through the wall of the ligated gut at several sites, starting at a fixed time after the Nembutal injection, so that all of the animals were at the same stage of anesthesia during the absorption period. The abdomen was then closed until 25 minutes later when the intestine was excised, sliced open, and washed with its contents into a 500 ml volumetric flask where the contents were precipitated using the zinc hydroxide procedure of Somogyi(12). An aliquot of the protein-free filtrate was analyzed according to the Nelson (13) colorimetric modification of Somogyi's (14) method for slowly reacting sugars. Absorption was determined as the difference between the amount of sugar administered and the amount recovered. The rate of absorption was calculated [after Cori(9)] as the coefficient of absorption: mg absorbed/100 g body weight/hour.

The data were analyzed statistically by the use of Student's t, a method which has been recommended for small samples. The standard deviation was calculated as:

$$\sigma_x = \sqrt{\frac{\Sigma x^2}{N} - \left(\frac{\Sigma x}{N}\right)^2}$$

The t was found from the formula

$$t = \frac{M_x - M_y}{\sqrt{\left(\frac{\sigma_x^2 N_x + \sigma_y^2 N_y}{N_x + N_y - 2}\right)\left(\frac{N_x + N_y}{N_x N_y}\right)}}$$

M representing the mean, and N the number of units in the samples. The value of t gives the level of confidence for the difference Mx—My, taking into account the size of the samples(15).

Results. The data are presented in Table I. The mean coefficients of glucose absorption were 116.6 for the control rats and 72.0 for the phlorhizinized animals, a decrease of 38.2%. The level of confidence of the experimental difference, determined by the use of Student's t, is over 99.9%, indicating that there was a very significant inhibition of glucose absorption by phlorhizin.

Galactose absorption was reduced from a mean coefficient of 101.2 to 55.7 by phlorhizination. This inhibition was as significant as that of glucose. The degree of phlorhizin inhibition of galactose absorption (44.0%) was roughly the same as that of glucose.

Mannose absorption was reduced from a coefficient of 19.9 to 14.6 by phlorhizin, an inhibition of 26.6%. The data indicate some phlorhizin inhibition although the statistical reliability of this difference is poor (level of confidence only 33.4%). Sorbose was normally absorbed at such a slow rate, 8.3 mg/100 g/hour, that the differences in reducing

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TABLE I. Coefficients of Absorption of 5 Hexoses for Phlorhizinized and Control Rats.

	Control rats				Phlorhizinized rats				Effect of phlorhizin	
Sugar	No. rats	Coeff. of absorp.	σ	% of glucose	No. rats	Coeff. of absorp.	σ	% of glucose	% of inhibition	Level of confidence of diff., %
Glucose	18	116.6 (77-171)*	28.5	100.0	15	72.0 (54.5-100)	19.1	61.8	38.2	99.9
Galactose	23	101.2 (35-170)	28.6	86.9	25	55.7 (2-126)	31.8	47.8	44.0	99.9
Fructose	13	49.3 (18-77)	20.0	42.4	14	63.4 (25-88)	17.5	54.3	—28.6	92.8
Mannose	6	19.9 (6-37)	10.9	17.1	5	14.6 (1.5-27.5)	9.1	12.5	26.6	†
Sorbose	6	8.3 (1-20)	7.5	7.1	5	0.9 (0-2.5)	0.9	0.8	90.0	†

* The figures in parentheses indicate the range of coefficients found.
† Statistical analysis of samples of this size would be unreliable.

substances were within experimental error range. The effect of the phlorhizin was exaggerated with the small number of animals used and statistical evaluation would be inadequate.

The results for fructose absorption were in striking contrast to those for the other 4 hexoses. The phlorhizinized rats absorbed fructose at a rate 28.6% greater than the controls, the drug appearing to have increased rather than inhibited fructose absorption. The statistical significance of this increase is not high (level of confidence 92.8%), but at least there is no doubt that the drug failed to inhibit fructose absorption.

Discussion. The relative order of rates of absorption in the control rats compares favorably to that reported by Cori(9) and by Verzár(16), as shown in Table II. The absorption of galactose averaged 13% less than that of glucose in these experiments, but statistical evaluation assigns a low level of confidence to this (91%). The absorption rates, while in nearly the same relative order as those reported by Cori for unanesthetized rats, were actually 30-40% lower. This, together with some preliminary observations not reported here, has led us to believe that Nembutal anesthesia exerts a general depression on hexose absorption without altering the relative rates of absorption of the sugars to each other.

TABLE II. Intestinal Absorption of Hexoses Referred to Glucose as 100%.

	These data	Cori(9)	Verzár(16)
Glucose	100	100	100
Galactose	87	110	115
Fructose	42	43	44
Mannose	17	19	33
Sorbose	7	—	30

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Gammeltoft and Kjerulf-Jensen(17) have suggested that phlorhizin inhibits hexose absorption by blocking some enzyme necessary for the phosphorylation, and hence absorption, of glucose, galactose, and fructose. They have further postulated that the enzyme inhibited is the phosphate donor, adenosinetriphosphatase, rather than the hexose phosphorylase. The failure of phlorhizin to inhibit fructose absorption in these experiments makes it seem much more probable that it is the phosphorylase that is inhibited by phlorhizin, and suggests that separate enzymes may exist in the mucosa for the phosphorylation of each of the hexoses. The fructose phosphorylase would appear not sensitive to the inhibitory action of phlorhizin. The existence of specific phosphorylating enzymes is further supported by the recent finding of such specific enzymes as fructokinase in muscle(18) and galactokinase in liver and yeast(19).

Summary. Phlorhizin has been found to

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inhibit the intestinal absorption by rats of glucose, galactose, and possibly of mannose and sorbose, but not of fructose. This may indicate the presence in the intestinal mucosa of a specific enzyme for fructose phosphorylation which is not inhibited by phlorhizin.

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Observations on the Toxin of Influenza Virus.* (18107)

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Henle and Henle(1) reported the demonstration of a toxin associated with influenza virus and later studied this factor more extensively(2-4). During an investigation in our laboratory of further toxic manifestations of the virus on the circulatory system of the host(5,6) difficulties in maintaining the toxin-producing properties of the virus were encountered; therefore, it was felt desirable to repeat and extend some of the experiments of Henle and Henle. Only the PR8 strain† of influenza A virus was used. With careful attention to conditions of storage of the virus, consistent production of toxin was attained. In general, our observations of the lesions produced by the toxin, the relation of toxin to other established virus properties, its production and stability and host factors affecting toxicity have confirmed and amplified the findings of Henle and Henle, with a few differences. In addition, the susceptibility of the ferret to influenza virus toxin was demonstrated.

Methods. Toxin of the influenza virus was produced by inoculating the allantoic sac of 10 to 11 day chick embryos with 0.15 ml of a 10^{-6} dilution of infected allantoic fluid in brain-heart infusion broth. The eggs were then incubated at 37°C for 48 hours, after which they were chilled before harvesting the allantoic fluids. The fluids were tested for bacterial contamination before pooling. The allantoic fluid was tested for toxicity by injecting 1.0 ml amounts intravenously into the tail vein of 12 to 15 g CFW mice. Dilutions for intravenous injection were made in normal allantoic fluid. When virus was purified and concentrated it was done by adsorption and elution from red blood cells followed by high speed centrifugation(5). Virus was tested for infectivity by injecting 0.15 ml of 10-fold dilutions of viral suspensions into 10 to 11 day embryonated eggs. The presence of virus in fluids harvested from these eggs was determined by the hemagglutination technic using the method of Salk(7) with the exception that the concentration of the chick red cell suspension used was 0.5 instead of 0.25%.

Experimental. As the Henles have shown, the intravenous injection of allantoic fluid containing PR8 virus produced toxic manifestations consisting of gross lesions of the liver, spleen, frequently the intestines, the kidney, and engorgement of pulmonary vessels without parenchymal consolidation. In our experiments, alterations in some or all of

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