

# Phlorizin Inhibition of the Insulin Expansion of the Galactose Space in the Eviscerate Rat\*

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(Received for publication, October 27, 1958)

Several reports have recently appeared which indicate that phlorizin may act at certain cell membranes to inhibit penetration of them by glucose. A recent study by Krane and Crane (1) showed that in slices of rabbit kidney cortex, phlorizin can inhibit the rate of active accumulation of galactose from the medium. Under anaerobic conditions or in the presence of an uncoupler of oxidative phosphorylation, galactose penetration into the slice occurred by simple diffusion, but, nevertheless, phlorizin inhibited the rate at which this equilibrium was obtained. Thus these workers believed that phlorizin was not affecting any of the reactions furnishing energy for an active sugar transport. Chinard *et al.* (2), using a rapid injection into the renal artery in the dog, presented evidence that phlorizin causes the luminal side of the tubular cells to become impermeable to glucose.

Two additional reports dealing with other tissues also indicate a cell membrane site of action for phlorizin. Crane *et al.* (3) showed that the rate of penetration of 3-methyl glucose into Ehrlich ascites tumor cells was decreased by phlorizin, although "no" phlorizin entered the cells. Wilbrandt (4), on the basis of extensive kinetic studies, put forth the hypothesis that phlorizin inhibits glucose transport across the red cell membrane by blocking an enzyme which cleaves glucose from a membrane carrier.

In view of these considerations indicating that phlorizin may act directly to block the mechanism by which monosaccharides penetrate cell membranes, rather than directly to block the source of energy for transport, it was believed that it would be of interest to determine whether phlorizin has any more general effect in the body on membrane permeability to sugars. We wondered, for instance, whether it might act in such a general way as to alter the permeability of muscle cells to sugars. To test this question, it was decided to utilize the observation of Levine *et al.* (5) who observed that in the eviscerated, nephrectomized dog insulin could expand the galactose volume of distribution (galactose space) from one approximating the extracellular fluid volume to one equalling the volume of total body water. It was found that although phlorizin itself had no effect on the galactose space in the eviscerate rat, under certain conditions phlorizin could inhibit strikingly the insulin effect of expanding the galactose space. The purpose of the present paper is to present the evidence supporting this conclusion and to discuss it in the light of a membrane site for phlorizin action.

\* Supported by a grant from the Life Insurance Medical Research Fund of New York.

† This work was performed during the tenure of a Life Insurance Medical Research Fund Postdoctoral Fellowship.

## EXPERIMENTAL

Measurements of galactose space were carried out in 300-gm. eviscerated, nephrectomized white rats. Functional evisceration was done by the method of Russell (6) under Nembutal anesthesia. Since it has been shown that galactose is not measurably metabolized in the eviscerate preparation (5), its volume of distribution in such an animal can be used to study changes in cell permeability to galactose. Blood glucose levels were maintained in all eviscerate animals by giving a solution of 25 mg. of glucose per 100 gm. of body weight subcutaneously each hour. The design of each experiment is included in the legends of Tables I and II below. The dose of phlorizin employed was selected because it approximates that which has been shown to cause complete blockage of glucose reabsorption in the kidney (7). All blood samples were drawn from the inferior vena cava into heparinized syringes and centrifuged. Plasma galactose was determined by the photometric method of Nelson (8) with yeasting of the filtrates and galactose standards (9). Plasma concentrations were divided by 0.94 to convert to plasma water concentrations. The volume of the galactose space was taken as the ratio of the amount of galactose administered to the concentration of galactose in the plasma water, and this is expressed as a per cent of total body weight.

## RESULTS

*Effect of Insulin on Galactose Space*—Table I shows data from an experiment which confirms the finding of Levine *et al.* (5) that insulin does, indeed, enlarge the galactose space. It is evident that this effect is apparent at 1 hour but is most marked at 2 hours. Thus at this time the control group of animals had a galactose space which was 28 ml./100 gm. of body weight, the approximate volume of the chloride space. In the insulin-treated animals, on the other hand, the galactose space was 45 ml./100 gm. of body weight, an increase of about 60 per cent above control levels. The magnitude of this increase in galactose space after insulin corresponds well with that found by Levine *et al.* in dogs. Since the eviscerate animal is essentially a muscle preparation, this effect has been interpreted as being due to an insulin action on the muscle cell membrane to facilitate galactose penetration (10).

*Effect of Phlorizin on Insulin Expansion of Galactose Space*—A preliminary series of experiments showed quite conclusively that phlorizin itself, in doses sufficient to produce maximal glycosuria in the rat, had no effect at all on the galactose space in the eviscerate preparation. Therefore, it was decided to carry out a second series of experiments to determine whether phlorizin affected the ability of insulin to expand the galactose space.

TABLE I

*Expansion of galactose space by insulin in eviscerate rat*

Galactose, 70 mg./100 gm. in volume of 0.5 ml. of 0.15 M NaHCO<sub>3</sub> per 100 gm. injected in inferior vena cava at zero time. Insulin (Squibb, regular), 3.2 units, injected subcutaneously at zero time. Two-ml. blood samples drawn at 1 and 2 hours for galactose determination.

Animal No.	Galactose space			
	Controls		Insulin	
	1 hr.	2 hrs.	1 hr.	2 hrs.
	<i>ml./100 gm. body weight</i>		<i>ml./100 gm. body weight</i>	
1	25.5	27.2	38.3	43.1
2	25.2	26.6	42.4	46.9
3	25.7	31.0	37.6	44.8
Mean . . . . .	25.5	28.3	39.4	44.9

TABLE II

*Inhibition by phlorizin of insulin-induced expansion of galactose space in eviscerate rat*

Galactose, 70 mg./100 gm. in 0.15 M NaHCO<sub>3</sub>, injected into inferior vena cava at zero time. In test animals this solution also contained phlorizin, 14 mg./100 gm. Volume of injected solution was 0.5 ml./100 gm. of body weight. At 30 minutes 0.4 unit of regular insulin (Squibb) was given intravenously and 2.8 units subcutaneously. Blood samples were drawn at 2 hours for galactose determination.

$t = 5.82$ ; level of significance of difference between means,  $p < 0.001$ .

Animal No.	Galactose space	
	Insulin	Insulin + phlorizin
	<i>ml./100 gm. body weight</i>	
1	51.7	35.4
2	48.4	30.6
3	59.4	46.1
4	59.3	37.4
5	45.1	45.1
6	58.4	41.8
7	55.7	31.3
8	46.9	30.2
9	51.1	33.7
Mean . . . . .	52.9	36.8
S.D. . . . .	5.52	6.20

In Table II are shown the results of these studies with nine animals in each group. The data show that the animals which received phlorizin and insulin had a significant ( $p < 0.001$ ) lower volume of distribution of galactose than the animals which received insulin alone. Thus the galactose space in the insulin-phlorizin group was 36.8 ml./100 gm. of body weight, a figure not much above the galactose space in the noninsulinized, non-

phlorizinized animal (Table I). However, the control group of animals, treated in an identical manner but not given phlorizin, had a mean galactose space of 52.9 ml./100 gm. of body weight. Thus it appears that phlorizin, in doses used here, is capable of blocking almost completely the now familiar insulin-induced expansion of the galactose space in the eviscerate rat.

It may be calculated that the dose of phlorizin given (14 mg./100 gm. of eviscerated body weight) would result in a maximal final phlorizin concentration of about  $2 \times 10^{-3}$  M if it were entirely limited to the extracellular fluid volume (as measured by the insulin space); or if it were distributed throughout the entire body water, it would result in a minimal concentration of about  $4 \times 10^{-4}$  M. Thus the concentration which blocks the insulin effect on galactose distribution is approximately in the range found to promote mitochondrial swelling in isotonic sucrose (11) as noted in the previous paper.

## DISCUSSION

The data presented here show that whereas phlorizin alone is not able to affect the penetration of galactose into cells, it is able to prevent in a rather striking way the facilitation of galactose penetration caused by insulin. The exact meaning of this observation is not obvious from the present data and will depend, no doubt, on a better understanding of the action of insulin. A speculative discussion of how insulin might alter the cell permeability to sugars has recently been presented by Krahl (12). Insulin is a polypeptide of known structure which may combine with the lipoproteins of the cell membrane and either alter the "pore" size and thus the diffusion permeability or alter the activity of a membrane "carrier" and thus facilitate active transport across the cell membrane.

That phlorizin itself is capable of combining with proteins on the surface of certain cells has recently been shown. Rodriguez and Osler (13) have found that the lysis of sensitized sheep erythrocytes by guinea pig complement is markedly inhibited by phlorizin in concentrations of  $10^{-2}$  M to  $10^{-4}$  M. The mechanism of this inhibition indicates a cell surface competition between phlorizin and one of the complements for the sites on the sensitized erythrocytes.

Whatever the ultimate explanation of the phlorizin effect in blocking an insulin expansion of galactose space, the observation adds another link to the chain of evidence supporting a membrane site for the action of this glycoside and provides another experimental tool for the investigation of the nature of its interaction with cell membranes. That its gross biological effects in the intact animal seem to be limited mainly to sugar transport in kidney and intestine remains an unsolved problem.

## SUMMARY

Experiments with the functionally eviscerate rat have shown that phlorizin, although without effect on the galactose space when given alone, is capable of preventing the usually observed expansion of this galactose space produced by insulin. This observation is presented and discussed in terms of a membrane-carrier site for the action of phlorizin in inhibiting the biological transport of sugars.

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