days; wind speed was higher and solar radiation was lower on two days; wind direction aloft (see *h*) was north of west on two days; no difference was found on one day.

Thus, dilution of precursor material for the photochemical reaction, insufficient irradiation, or wind direction aloft from a quarter other than Washington may account for the findings on 10 of the 11 days when winds at the surface were southerly but ozone levels were low at Beltsville.

While the weather parameters on the remaining day (16 September) appeared to be similar to those for the five high-ozone days, the 0600 sounding at Silver Hill, Md. (*7*), differed; instead of a surface-based inversion, the sounding showed a 500-ft-deep isothermal layer, with an inversion immediately above, and southwest wind of 22 knots 700 ft above the surface. The temperature in the tobacco field at 1152, the time of peak relative ozone level (34), was the highest in the period of ozone record (97°F). While the velocity rate of ozone decomposition increases rapidly with temperature (*8*), it is believed that the antecedent high wind speed aloft was the primary limiting factor in this case.

Weather-fleck injury to tobacco, of the kind previously reported at Beltsville in association with high ozone levels (*1*), was observed northeast of Hartford, Conn., on 15 Sept. 1958 (*9*). Physiological insult by ozone was presumed to have occurred the day before. Weather parameters applicable to the Hartford area on this day were found to be similar to those associated with instances of high ozone level at Beltsville (Table 1). Likewise it was found that the weather parameters for Washington, D.C., on 14 September, fell into the same ranges, if, as was assumed, the maximum ozone level occurred about 1030 (the ozone recorder was not operated on that day). Whether fleck was observed at Beltsville the following day (*1*). While the test data indicate the ranges within which certain weather parameters make possible the occurrence of high-ozone levels downwind of a metropolis, further work is necessary to establish critical limits (*10*).

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Molecular Structural Factors in Competitive Inhibition of Sugar Transport

Abstract. The high potency of phloretin as a competitive inhibitor of the human red cell's monosaccharide transport system is not shared by any of several molecular fragments of phloretin, but is duplicated in certain artificial estrogens resembling phloretin in respect to the spacing between terminal phenolic —OH groups. Related molecules which are slightly less extendible are comparatively inactive.

The glucoside phlorhizin, the classical agent for inducing experimental glycosuria through inhibition of sugar reabsorption in the renal tubules, similarly interferes with the passage of monosaccharides through the surface of human erythrocytes (*1*) and mouse ascites tumor cells (*2*). But the glucose moiety of the phlorhizin molecule does not appear to be involved in this action, since (at least in the red cell system) slowing of sugar transfer is much more pronounced with the aglucon (phloretin) than it is with the glucoside (*3*). The systematic manner in which the degree of this inhibition is determined by the sugar and phloretin concentrations (*4*) accords well with the mass action law as applied to a case of direct competition between inhibitor and substrate (sugar) for some "carrier" site on the cell surface.

A novel type of substrate stereospecificity has recently been reported for this sugar-transfer system (*5*), such that the critical requisite for reaction with aldoses is evidently the energetic stability of the sugar in the particular pyranose "chair" conformation designated as "C1." But both rings of the phloretin molecule are aromatic (hence essentially planar), and neither assumes any of the conformations in which the substrate sugar rings could be stable. Therefore a quite different factor must underlie the even tighter association with phloretin which is implied by the inhibition kinetics. Hence the present study (*6*) was directed toward identification within the phloretin molecule of the atomic groupings critical to the high, specific inhibitory potency, in the hope of developing a clue about the physicochemical structure at the hypothetical carrier sites.

To this end, various agents (*7*) were compared as inhibitors of the red cell's monosaccharide transport system, principally in terms of decrease in the rate of egress from the cells of D-glucose; as illustrated in earlier reports (*4, 8*), estimation of rates is simpler with this procedure than with "entry" experiments. Washed human erythrocytes equilibrated with the sugar at about 0.15*M* were transferred, at 37°C, to a much larger volume of sugar-free medium. The relatively slow sugar exit which ensues is accompanied by rapid osmotic equilibration of the water, and the resultant cell shrinkage was followed in the very dilute cell suspension by continuous optical densitometry (method of Ørskov, *9*). The usual medium was a mixture of the chlorides of Na, K, Ca, and Mg in a molar proportion of about 150:6:3:2 and at a total tonicity of 300 to 305 milliosmole/lit. when buffered at *p*H 7.4 with 32m*M* tris(hydroxymethyl)-aminomethane.

A priori, the length of the phloretin molecule raises the likelihood that activity might persist in the absence of one end or the other. But examination of assorted fragments from each end of the phloretin structure (the two columns of the upper section of Fig. 1) has revealed no agent of comparable potency; and the residual activity shown at higher concentrations appears to be independent of sugar concentration, so that it cannot be based on competition with the substrate. Moreover, when a combination of overlapping moieties (phloretic acid and phlorpropiophenone) was used, only a direct additivity of the separate inhibitory effects was observed, with no appreciable synergism. The much higher potency of the intact molecule thus focused attention on the orientation and spacing of the terminal groups. Simple α,ω-dihydroxyl derivatives of hydrocarbons of similar length (nonamethylene glycol or decamethylene glycol) were almost totally inactive; but among other diphenolic forms (lower section of Fig. 1), certain ones proved to be extremely potent in slowing sugar transfer.

The most effective inhibitor found was the synthetic estrogen, diethylstilbestrol; at physiological *p*H, it was about half again as potent as phloretin, while its saturated homologue, hexestrol (not quite so potent an estrogen) was some-

what weaker than phloretin. Since phloretin itself also shows definite estrogenic activity (*10*), some correlation of these two biological properties might be pursued. However, the related powerful estrogen dienestrol is relatively inert in the red cell system. Still less active is naringenin, which differs structurally from phloretin only in elimination of two hydrogen atoms, leading to assumption of the flavanone form given in Fig. 1. Some consistent structural distinction was therefore sought between these two rather inert materials and their active counterparts. In line with the implication of a two-point requirement as developed above, attention is called to the spacing of the terminal phenolic —OH groups. On the basis of either the Fisher-Hirschfelder-Taylor or the Catalin molecular models, all three of the especially active agents permit a center-to-center spacing of well over 13 A between the terminal hydroxyl O-atoms apposed to a flat surface. But with the two comparatively ineffective homologs this degree of separation cannot be achieved—in the case of naringenin, because of the shortening imposed by the internal bridge, and in the case of dienestrol because steric restraints make it necessary for the molecule to fold somewhat in bringing the terminal groups against a flat surface. In the same vein, it may be noted that the most active molecule, stilbestrol, is unique among the group in being held rather stiffly at full extension by reason of the double bond at the center.

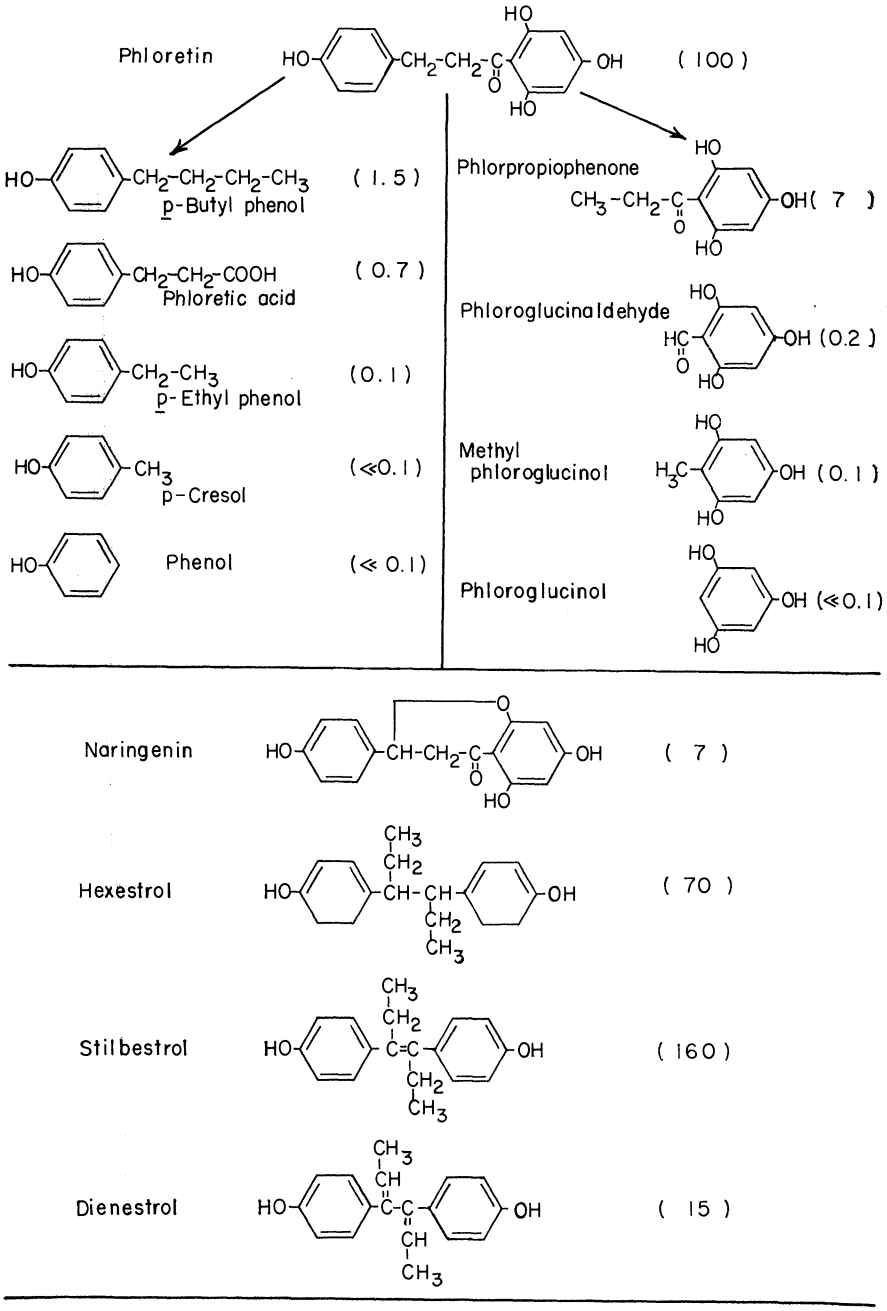

Fig. 1. Comparison of test agents as inhibitors of sugar transport through the surface of human red cells. Potency ratings in parentheses are reciprocals of approximate millimolarity at a level producing 50-percent inhibition in the test system described in the text.

It is therefore proposed that groups capable of reversibly binding or associating with phenolic —OH groups may be distributed in a recurrent spacing pattern over part, or all, of the red cell surface, and that the operation of the sugar-transfer system involves these same loci. The specially high potency of certain of the diphenolic agents would then be interpreted as deriving from the fact that their particular molecular geometry allows them to fix at two points on this matrix, with the corresponding large gain in tenacity familiar in the context of ion-exchange and chelation chemistry.

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