PHYSIOLOGY

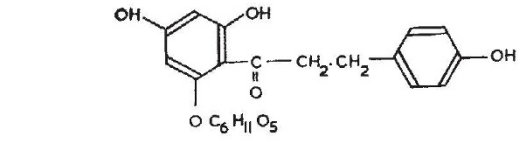
Polyphenolic Compounds and Intestinal Transfer

It is well established that phlorrhizin and its aglycone phloretin affect hexose transfer in a number of tissues. Recently Forsling and Widdas[1] showed that phenolphthalein caused competitive inhibition of facilitated glucose transfer in human erythrocytes. In view of its purgative action, it was of interest to test whether phenolphthalein also affected intestinal transfer mechanisms. At the same time the opportunity was taken to test another polyphenolic compound phenol red (phenolsulphonphthalein), a molecule of similar structure which is known to be transferred by the renal tubule. The formulae of these compounds are shown in Fig. 1.

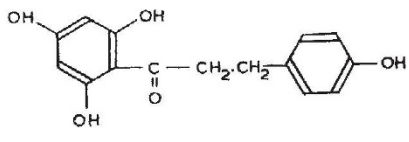
Experiments were carried out with sacs of everted small intestine of the rat, as used by Barry, Matthews and Smyth[2], and the middle fifth of the combined jejunum and ileum was used in all cases. Glucose (28 mM) or methionine (15 mM) was initially present in the mucosal fluid and the transfer of these substances was investigated in the presence and absence of the polyphenols. The results are shown in Table 1.

In confirmation of Parsons, Smyth and Taylor[3], phlorrhizin (5×10^{-5} M) strongly inhibited glucose transfer, and at 5×10^{-4} M nearly abolished glucose transfer. As was found by Jervis, Johnson, Sheff and Smyth[4], phloretin (5×10^{-5} M) caused little inhibition, but we have now found that at 5×10^{-4} M it markedly reduced glucose transfer. Phenolphthalein (5×10^{-5} M) had almost as great an effect as equimolar phlorrhizin on glucose transfer, while phenol red even in a concentration of 10^{-4} M had little effect.

Any substance inhibiting transfer of glucose from the mucosal fluid may produce its effect either on glucose entry or on metabolism. These two activities can be separated by using glucose in the serosal fluid, as the glucose can then be metabolized without going through the entry mechanism. The effect of the inhibitor can be examined either by measuring fluid transfer (which depends on glucose metabolism) or by disappearance of glucose from the system. When glucose was present initially in the serosal fluid, phenolphthalein (5×10^{-5} M) did not affect glucose disappearance, and had only very slight, if any, effect on fluid transfer. Hence its action must be to prevent glucose from entering the cell from the mucosal side. Phloretin, however, reduced fluid transfer from 2·3 ml. to 0·4 ml., and this suggests that glucose metabolism was being impaired.



PHLORRHIZIN



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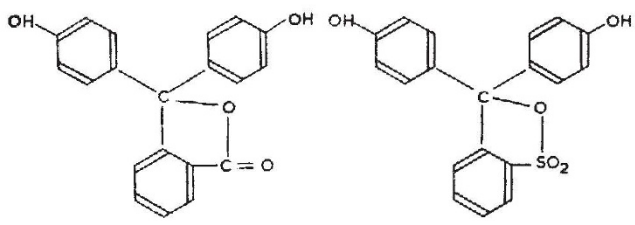

PHENOLPHTHALEIN PHENOLSULPHONPHTHALEIN

Fig. 1

Table 1

Polyphenolic compound	Intestinal transfer Glucose (μM)	Methionine (μM)
None	337 ± 9(12)	61 ± 4 (10)
5×10^{-5} M Phlorrhizin	102 ± 7 (6)	62 ± 1 (7)
5×10^{-4} M Phlorrhizin	18 ± 3 (5)	41 ± 2 (6)
5×10^{-5} M Phloretin	307 ± 10 (6)	53 ± 4 (6)
5×10^{-4} M Phloretin	94 ± 6 (9)	22 ± 1 (6)
5×10^{-5} M Phenolphthalein	131 ± 12 (6)	36 ± 3 (8)
1×10^{-4} M Phenol red	317 ± 19 (6)	66 ± 3 (6)

Values given are the means ± *S.E.* The figures in parentheses indicate the number of experiments performed.

The effect of phenolphthalein is less specific than that of phlorrhizin as it inhibited methionine transfer in a concentration of 5×10^{-5} M, while a much greater concentration of phlorrhizin was required to cause a similar inhibition. Phenol red was also ineffective on methionine transfer in the concentrations tested. Phloretin (5×10^{-4} M) had more effect on methionine transfer than phlorrhizin in the same concentration, and this could be because its greater lipid solubility permitted easier entry into the cell with more effect on metabolism.

The difference between phenol red and phenolphthalein is of great interest on account of the similarity in their structure. A possible explanation is the difference in pK values. At a pH of 7·4 phenolphthalein will be mainly in the undissociated form, while phenol red will be largely dissociated, and the undissociated form may be the effective inhibitor of the entry process.

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[1] Forsling, M. L., and Widdas, W. F., *J. Physiol.*, **178**, 12P (1965).
[2] Barry, B. A., Matthews, J., and Smyth, D. H., *J. Physiol.*, **157**, 279 (1961).
[3] Parsons, B. J., Smyth, D. H., and Taylor, C. B., *J. Physiol.*, **144**, 387 (1958).
[4] Jervis, E. L., Johnson, F. R., Sheff, M. F., and Smyth, D. H., *J. Physiol.*, **134**, 675 (1956).

Comparative Study of the Urease in the Rumen Wall and Rumen Content

In the course of the ruminohepatic nitrogen cycle[1,2], or by supplementation in feeding[3], a considerable amount of urea can enter the rumen. The ureolytic activity of the rumen content was investigated and found to be due to some species of ruminal micro-organisms. Attempts to obtain enzyme preparations free from bacteria for a more accurate study, however, were unsuccessful[4]. Further investigations revealed that the urease activity of the ruminal mucosa appeared to be too high to be simply explained by surface contamination by rumen contents[5]. This communication deals with a study of partially purified urease preparations obtained from ruminal bacteria, as compared with partially purified enzyme prepared from the rumen mucous membrane, and the origin of the latter enzyme is also discussed.

Preparation of cell-free extracts. Washed suspensions of ruminal bacteria were prepared from the rumen content of freshly slaughtered cattle[6]. The bacterial cells were broken up using an M.S.E. ultrasonic disintegrator for 15 min. Cell-free extracts were obtained by centrifugation at 75,000*g* for 30 min. The mucous membrane from the rumen of freshly slaughtered cattle was washed thoroughly in a stream of cold water. The villi were cut off, homogenized in two parts of 0·1 M *tris*–HCl buffers (pH 8·5)