

the less, physically independent. This fact in no way depends upon having a single-step mechanism. If solute molecules are shuttled laterally by two independent diffusion processes, it can be shown⁴, as expected, that the diffusion coefficients add in the denominator, one reducing the effect of the other. In close analogy with eddy diffusion, both diffusion processes "act independently to exchange molecules between fast- and slow-moving regions". It is, in fact, possible to assign an effective coefficient of lateral diffusion (even though lateral diffusion, as such, is not necessarily involved) to describe the effect of velocity variations along a streamline. A close analysis of this shows that the effective diffusion coefficient varies with the distance across the interstice while the molecular diffusion coefficient is constant⁴. At any point these two are additive in the denominator, but the addition of the averages of the two, and this is essentially what was done in the earlier treatment³, is an approximation. I am at present looking for an improvement of the admittedly approximate derivation previously given.

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¹ Klinkenberg, A., and Sjenitzer, F. (previous communication).

² Giddings, J. C., *J. Chem. Phys.*, **31**, 1462 (1959).

³ Giddings, J. C., *Nature*, **184**, 357 (1959).

⁴ Giddings, J. C., *J. Chromatography* (in the press).

BIOCHEMISTRY

Exclusion of Inulin from Solutions of Hyaluronic Acid

THE partition of inulin between buffer and solutions containing hyaluronic acid has been observed by dialysing inulin to equilibrium between the two solutions. 'Millipore HA' membranes were used which are readily permeable to inulin but impermeable to hyaluronic acid. The hyaluronic acid was prepared from ox synovial fluid by the method of Ogston and Stanier¹; inulin was Kerfoot biochemical reagent twice 'recrystallized' from dilute ammonia.

The partition depended on the concentration of hyaluronic acid according to $\log K = 2w$, where K is the ratio of concentration of inulin in buffer to that in the hyaluronic acid solution at equilibrium and w is the weight of hyaluronic acid/100 ml. The partition was independent of the concentration of inulin, the direction of equilibration, pH and ionic strength. Thus at $w = 0.2$, $K = 2.5$, and this can be thought of as the exclusion of inulin from 60 per cent of the volume of the hyaluronic acid solution. These results have a bearing on the use of inulin for determining the volumes of tissue spaces.

The effect is not peculiar to inulin, but is shown by other carbohydrates and by proteins, to degrees depending on their effective molecular volumes; this suggests that the mechanism of exclusion is steric.

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¹ Ogston, A. G., and Stanier, J. E., *Biochem. J.*, **46**, 364 (1950).

Alkaline C-1 Fructose-1,6-diphosphatase : Evidence for its Participation in Photosynthesis

PATHWAYS proposed for the conversion of carbon dioxide to hexose monophosphate during photosynthesis require the cleavage of the phosphate linked to carbon-1 of either fructose-1,6-diphosphate, or sedoheptulose-1,7-diphosphate, or both¹⁻³. A C-1 alkaline phosphatase specific for fructose-1,6-diphosphate has been prepared from spinach leaves and characterized by Racker and Schroeder³. This enzyme was not thought to participate in photosynthesis since it did not appear to be localized in the chloroplasts. Recently it was reported⁴ that pea chloroplasts isolated in various aqueous media lost about 90 per cent of their ribulose-1,5-diphosphate carboxylase, an enzyme considered to play an essential part in photosynthesis¹. Since this finding indicated that photosynthetic enzymes were not necessarily retained in chloroplasts isolated by the usual procedures, the possible function and intracellular distribution of alkaline fructose-1,6-diphosphatase in photosynthetic tissues were re-investigated.

A survey of a number of plants and bacteria disclosed that alkaline fructose-1,6-diphosphatase was widely distributed in photosynthetic tissues. In addition to spinach³ and pea⁵ leaves, alkaline fructose-1,6-diphosphatase has been demonstrated in cell-free extracts of photosynthetic tissues from barley, alfalfa, *Spirodela*, the fern *Pteris gautheri*, the algae *Euglena gracilis* and *Chlamydomonas reinhardtii*, and the photosynthetic bacteria *Rhodospirillum rubrum* and *Chromatium*. Conversely, the enzyme was not found in extracts of pea root or an apoplastidic strain (streptomycin-bleached) of *Euglena gracilis*. The activity of the enzyme in etiolated pea leaves was less than 5 per cent of the maximum activity found in green pea leaves. In related work in which quantitative measurements were made of the levels of various enzymes in growing pea leaves, it was observed that the alkaline fructose-1,6-diphosphatase activity of the leaf followed a pattern of development similar to that previously ascertained for a number of photosynthetic enzymes⁶. The above evidence attests to a close correspondence between alkaline fructose-1,6-diphosphatase and chloroplast development and function.

Intracellular distribution studies of cell particulates isolated in either 0.5 M sucrose or 0.35 M sodium chloride were inconclusive because of the apparent loss of most of the photosynthetic enzymes from the chloroplasts during the fractionation procedure. However, this work did show that a small amount of

Table 1. THE DISTRIBUTION OF ALKALINE FRUCTOSE-1,6-DIPHOSPHATASE IN PEA LEAF AND *EUGLENA* FRACTIONS ISOLATED IN NON-AQUEOUS MEDIA

Plant material	Density of isolated fraction	Fructose-1,6-diphosphatase*	
		Per cent activity in fractions	Activity per chlorophyll (μ moles P/m.in./ μ gm. chlorophyll)
Pea leaf	< 1.34	68	0.52
	1.34-1.40	28	0.57
	> 1.40	4	0.55
Autotrophic <i>Euglena</i>	< 1.29	88	0.32
	1.29-1.36	9	0.57
	> 1.36	3	0.57

* The reaction mixture contained *tris*(hydroxymethyl)aminomethane buffer, pH 8.5 (100 μ moles/ml.), fructose-1,6-diphosphate (3.5 μ moles/ml.), magnesium chloride (5 μ moles/ml.), and ethylenediamine tetraacetate (1.6 μ moles).