



Fig. 2. ○, *E. coli* (refs. 5 and 6); □, yeast (ref. 7); △, calf liver (ref. 3); ■, pea seedling (refs. 4 and 8); ●, reticulocyte of rabbit (ref. 9)

The value of c assumed here means that, in the ribosome, an atom or an atomic group with the atomic weight of 14 (such as N or $-\text{CH}_2-$) is on an average contained in the volume of $(4 \text{ \AA})^3$. If the ribosomal particle were really a closely packed solid molecule as supposed from the electronmicroscopic observations, such a value of c would not seem so extraordinary or unreasonable. Thus it might be concluded that RNA should be compactly folded up in the ribosome, which is, in contrast to the isolated RNA, hydrodynamically approximated by a solid sphere, and the isolated RNA by a rigid spherical one composed of matted Gaussian chains or those similar to them, but without free drain. Moreover, the relations (2) or (6) would be available for estimating the approximate value of molecular weight of a ribosomal RNP by its ultracentrifugal data only as (1) with the isolated RNA.

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Maltose in Red Clover (*Trifolium pratense*) Leaves

TRACES of maltose in leaf extracts are generally attributed to amylase action on starch during processing of the samples and are avoided if enzymes are inactivated immediately after sampling. During investigations of diurnal fluctuations in red clover (*Trifolium pratense*) leaf carbohydrates, however, we have obtained the following evidence that free maltose is present in leaves collected at night.

Leaf samples (25 g wet wt.) collected at intervals over 24 h, during periods of sunny, summer weather (day-break, 5-5.30 a.m.; darkness, 7.30-8 p.m.), were plunged immediately into boiling ethanol and processed to soluble sugar extracts¹. Chromatograms of the extracts showed an easily detected reducing component, corresponding to maltose, in the night samples; this component was not detected in the day samples or in similar extracts of day

or night samples of stems and petioles. The reducing disaccharide (20 mg), isolated by fractionation on charcoal-'Celite' of the extract from 100 g (wet wt.) of leaves collected at 1 a.m., was identified as maltose as follows. On chromatograms developed with a variety of solvents it had the same R_G as maltose and was clearly different from other diglucoses including cellobiose, while with diphenylamine-aniline it gave the blue colour typical of a 1-4 linked reducing aldohexose disaccharide². The sugar also had $\alpha_D + 131^\circ$ (maltose monohydrate, $\alpha_D + 130.4^\circ$), yielded glucose only on acid hydrolysis and gave an osazone with the characteristic crystalline form of maltosazone. The maltose-level in the leaves was measured by quantitative paper chromatography of the leaf extracts³, using aniline hydrogen phosphate reagent, after first hydrolysing sucrose with an invertase which did not attack maltose. Similar results were obtained each time samples were collected; in a typical run the maltose (dry wt. basis) rose steadily from 30 mg per cent at 8 p.m. to 120 mg per cent at 5 a.m., after which it declined to an undetectable level by 7 a.m.

Enzyme extracts, prepared by grinding clover leaves in citrate buffer (0.1 M, pH 6.0), centrifuging and dialysing, were incubated with amylose and maltose respectively at various pH values and the digests analysed at intervals, by paper chromatography, for liberated sugars. Amylose digests showed a rapid release of maltose (pH optimum, 4.0-6.5) followed by a slower release of glucose, while the maltose digests showed a slow release of glucose (pH optimum 5.5-6.5). It is possible, therefore, that the maltose found in the leaves results from the different rates of activity of these two enzymes provided they are involved in the degradation of chloroplast starch at night⁴. Disappearance of maltose during the early hours of daylight may be due to continuing maltase action or to the use of the maltose as a primer in subsequent starch resynthesis.

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Carbohydrases of the Rumen Oligotrich *Eremoplastron bovis*

FROM small-scale, pure cultures of several rumen oligotrichs, including *Eremoplastron bovis* (*Eudiplodinium neglectum*, Dogiel¹), Hungate² prepared extracts containing cellulase and cellobiase. The extracts were not tested for other carbohydrases and appear to represent the only direct evidence for cellulolytic enzymes in rumen protozoa, apart from a recent observation of cellodextrinase in the extract from mixed *Entodinium* species³. Cultures of several oligotrich species were recently established⁴ in this laboratory, enabling us to prepare 2-3 g (wet wt.) of cells of *Eremoplastron bovis* for a more detailed examination of its carbohydrases.

The *E. bovis* cells were collected from a clonal culture⁴, freed from external bacteria, ground with Ballotini beads, diluted with water and centrifuged to yield extract A (10 ml.). The debris was extracted successively with citrate and phosphate buffers (0.1 M, pH 6.0 and 7.0 respectively) to yield extracts B and C. Extracts were dialysed, at 2° against water or buffer, and tested for carbohydrases in digests³ which were incubated under toluene for 48 h and analysed at intervals for liberated sugars by paper chromatography. Extract A rapidly hydrolysed starch, xylans, xylodextrins and cellodextrins, hydrolysed maltose, xylobiose, cellobiose, laminaribiose,