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SHORT COMMUNICATION

# PHOTOSYNTHETIC CARBON DIOXIDE FIXATION BY ISOLATED CHLOROPLASTS IN GOOD'S BUFFERS

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**Abstract**—Carbon dioxide fixation by isolated chloroplasts, in the presence of tris (Tris(hydroxymethyl)-aminomethane, or 2-amino-2-hydroxymethylpropane-1,3-diol), was compared with that in tricine (N-tris (hydroxymethyl)methylglycine), HEPES (N-2-hydroxymethylpiperazine-N'-2-ethanesulphonic acid) and TES (N-tris(hydroxymethyl)-methyl-2-amino ethane sulphonic acid). Initially, the rates were similar but subsequently a progressive decline in rate developed in the presence of tris which was not observed with the other buffers.

## INTRODUCTION

TRIS (Tris(hydroxymethyl)aminomethane, or 2-amino-2-hydroxymethylpropane-1,3-diol) is widely used as a pH 7–9 buffer in biochemical reactions. It has been preferred to phosphate buffers in studies with isolated chloroplasts because (*a*) it is not a metabolite, (*b*) it does not precipitate certain cations as insoluble salts, and (*c*) it was thought to be largely non-inhibitory.

Good[1] introduced tricine (N-tris(hydroxymethyl)methylglycine) in work on the Hill reaction. Its advantages over tris as the buffering agent were two-fold. The number of anions present in tricine buffers is smaller than that in tris buffers (and therefore the uncoupling of photophosphorylation from oxygen production is lessened) and the sole inhibitory action of tricine appeared to be osmotic since the effects of high concentrations of tricine were paralleled by the effects of high concentrations of sucrose.[1] This paper reports the results of experiments in which carbon dioxide fixation by isolated chloroplasts in tris was compared with that in tricine, and two other buffers, TES (N-tris(hydroxymethyl)methyl-2-amino ethane sulphonic acid) and HEPES (N-2-hydroxymethylpiperazine-N'-2-ethanesulphonic acid), developed in Good's laboratory.[2]

## RESULTS

Table 1 compares photosynthetic $CO_2$ fixation by isolated chloroplasts in the presence of tris, tricine, HEPES and TES after three time intervals. Initially, fixation was the same in all four buffers but after more prolonged incubation it will be seen that there was a decline which was most marked in the presence of tris-HCl.

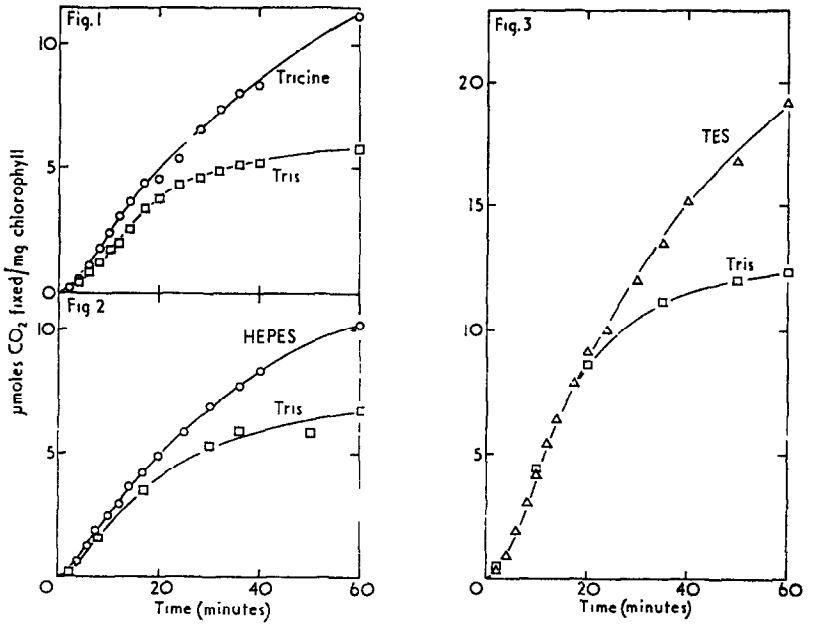
Figures 1, 2 and 3 are complete progress curves (for three separate chloroplast preparations) in which $CO_2$ fixation in tris is compared with that in the other buffers as before. The

[1] N. E. GOOD, *Arch. Biochem. Biophys.* **96**, 653 (1962).
[2] N. E. GOOD, G. D. WINGET, W. WINTER, N. T. CONOLLY, S. IZAWA and R. M. M. SINGH, *Biochemistry* **5**, 467 (1966).

initial lag is a characteristic of the system which has been reported elsewhere.[3, 4] It is again apparent that fixation declined more rapidly in the presence of tris than in the presence of the other buffers, though it must be emphasized that these results give no indication of the extent to which the changes in the ionic composition of the medium may be responsible for this effect.

TABLE 1. CARBON DIOXIDE FIXATION IN DIFFERENT BUFFERS AS A FUNCTION OF TIME

| Time (min) | μmoles $CO_2$/mg chlorophyll | | | |
|---|---|---|---|---|
| | Tris | Tricine | HEPES | TES |
| 5 | 0·56 | 0·55 | 0·60 | 0·57 |
| 15 | 2·59 | 3·32 | 3 47 | 3·88 |
| 40 | 5·03 | 10·73 | 8 71 | 11·24 |




FIGS. 1, 2 and 3. PROGRESS CURVES FOR PHOTOSYNTHETIC CARBON DIOXIDE FIXATION BY ISOLATED CHLOROPLASTS IN TRIS-HCl COMPARED WITH TRICINE-NaOH (FIG. 1), HEPES-NaOH (FIG. 2) AND TES-NaOH (FIG. 3) ALL AT pH 7·5. EACH REACTION MIXTURE CONTAINED THE ADDITIVES LISTED UNDER "EXPERIMENTAL". CHLOROPHYLL 46 μg (FIG. 1); 42 μg (FIG. 2); 33 μg (FIG. 3).

## DISCUSSION

Tris has proved to be a buffer of remarkable usefulness in a wide variety of biochemical systems. In the isolation of chloroplasts alone, the term "Tris-Na-Cl" has become accepted jargon for a medium which has contributed to work of great importance. However, in the absence of acceptable alternatives, the continued use of tris could conceivably stultify further

[3] D. A. WALKER, *Plant Physiol.* **40**, 1157 (1965).
[4] D. A. WALKER, Proc. NATO Advan. Study Inst. *Biochemistry of the Chloroplast*, Aberystwyth, 1965, Vol. 2. Academic Press, New York (In press).

improvements in techniques and its very ubiquity might encourage too ready acceptance of phenomena which might be artefacts of its presence. The work by Good and his colleagues[1, 2] in producing new buffers is therefore extremely welcome. Our present experience is that photosynthetic carbon dioxide fixation by isolated chloroplasts declines less rapidly in tricine, HEPES and TES (brought to pH 7·5 with NaOH) than in tris (adjusted with HCl). Of these, tricine has the added advantage that it is easily prepared from readily available reagents.

## EXPERIMENTAL

Pea seedlings (*Pisum sativum* var. Laxton Superb) were grown, and chloroplasts prepared, as described previously (Walker[3,5]). The chloroplasts were suspended finally in 0·45 M sucrose containing 0·1% NaCl, 0·1% $MgCl_2$ and 0·005 M sodium isoascorbate but no buffer.

Tricine was prepared by the method of Good[1] and twice recrystallized from aqueous ethanol. Samples of TES and HEPES were kindly given by Dr. N. E. Good.

Carbon dioxide fixation was estimated using $^{14}CO_2$ as before.[3,5] Reaction mixtures contained 0·1 ml of chloroplast suspension (about 0·04 mg chlorophyll) in a final volume of 0·3 ml containing 0·5 $\mu$mole $Na_2HPO_4$, 0·25 $\mu$mole $MgCl_2$, 0·5 $\mu$mole $MnCl_2$, 0·5 $\mu$mole EDTA. 1 $\mu$mole reduced glutathione, 2 $\mu$moles ribose-5-phosphate, 2·95 $\mu$moles carbonate–bicarbonate mixture (including either 20 $\mu$c or 50 $\mu$c $^{14}C$) and 7·5 $\mu$moles of the appropriate buffer adjusted to pH 7·5 with NaOH or HCl. Following illumination in saturating light at 20°, acidified samples were dried on lens-tissue discs on aluminium planchettes and their radioactivity determined with a Nuclear Chicago gas-flow counter.

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[5] D. A. WALKER, *Biochem. J.* **92**, 22c (1964).