Grigg, G. W. Notes on competitive suppression and mutation assay.

That prototrophs may be suppressed by large numbers of auxotrophs sharing a common medium is no doubt familiar to Neurospora workers. This effect, unless controlled, can bias results in mutation experiments involving screening for prototrophic reversions from auxotrophy. The mechanism of the effect is known (Grigg 1958 Austr. J. Biol. Sci. 11:69). It is due to the removal of the carbohydrate energy source from the selection medium by the non-growing auxotrophic population. The medium may be virtually exhausted of its carbohydrate source before the prototrophic cells can multiply to microscopic colony size, in which case they will not be detected. Whether or not a prototrophic conidium will grow to form a colony depends on several factors: (1) the growth rate of the prototroph, (2) the rate of exhaustion of the medium by the auxotroph - this may vary considerably between treated and control cell populations, and (3) the initial concentration of the energy source.

Factors 1 and 3 may be fixed by the parameters of the experiment. Factor 2 can usually be varied. It in turn is dependent on the concentration of auxotrophic cells, on the constitution of the medium and on the experimental treatment the auxotrophs experience. A mutagen which is toxic or is a metabolic inhibitor may considerably decrease the rate of uptake of the energy source. On the other hand, some other substances, such as caffeines, may stimulate the rate of sugar uptake at concentrations of the purine which are scarcely toxic. Inhibitors tend to decrease the suppression by slowing down the exhaustion of the medium, whereas stimulants increase it and thereby appear as less effective mutagens than they really might be.

Several variables can be adjusted to limit the extent of suppression. These include decreasing the concentration of auxotrophs or increasing the amount of carbohydrate in the medium. There is, however, a simple way in which competitive suppression could be completely prevented in Neurospora mutation experiments. This could be achieved by using mutation of an amino acid dependence to independence as the assay system and a carbohydrate in the selection medium which, to be utilized, requires enzymatic induction. The auxotrophic conidia grown up on glucose and, hence, non-adapted to the carbohydrate of the selection medium, would need to be starved of the dependent amino acid to prevent enzyme synthesis from occurring. Prototrophic reversions, which would not be amino acid starved should synthesize the required adaptive enzyme and utilize the carbohydrate for growth. Since the non-adapted auxotrophs are unable to utilize the carbohydrate, they would not remove it from the medium and thus would not suppress the prototrophic conidia. Should several post-mutagenic treatment cell-replications be desirable and the limiting amino acid be added to the selection medium to permit these, an amount of glucose should also be added in excess of that required for the replications to prevent adaptation to the alternative carbohydrate present in the medium.

Although this technique of avoiding competitive suppression has not been tested in Neurospora as yet, we have found it to be effective with a try$^-$, lac$^+$ strain of E. coli (Grigg Nature, in press). The cell

population was grown up on glucose so as to be deficient in ß-galactosidase and unable to utilise lactose, the sole carbohydrate of the medium. Production of new enzyme could not proceed because the cells were starved of tryptophan. Prototrophs could synthesize new ß-galactosidase and, hence, could use the lactose of the medium for growth. Using this technique, we found that cell concentration 100x greater than thos allowable on glucose media could be safely used in screening or selection experiments without the intervention of competitive suppression effects. - - - C.S.I.R.O., Division of Animal Genetics, Sydney, N.S.W., Australia.