BIOCHEMICAL LETTERS

Determination of tryptophan oxygenase activity in rat liver homogenates

We have recently demonstrated that the assay of the liver enzyme tryptophan oxygenase (L-tryptophan:oxygen 2,3-oxidoreductase, EC 1.13.11.11) might be influenced by (1) a substrate-independent (blank) reaction interfering with the reading of kynurenine absorbance at 365 nm in samples from fed rats, (2) blood remaining in the liver affecting the amount of holoenzyme measured, and (3) rapid and progressive activation of the enzyme occurring *post mortem* in undisrupted liver tissue (Stowell & Mørland, 1983). Recently points (1) and (2) were subjected to some criticism (Badawy *et al.*, 1983).

The authors could not reproduce our findings with regard to point (1), and suggested that this could be due to a difference in carbohydrate content between their diet (45% w/w) and ours (50% w/w). We find it hard to believe that this small difference could have such importance, since the main carbohydrate of our diet, starch, gives less marked 'blank' reactions immediately after food consumption than does intake of glucose and sucrose solutions (Mørland, 1974). In our study (Stowell & Mørland, 1983) we demonstrated that the blank reaction was most marked during the first period after the animals had finished eating during the dark period. Thus the phenomenon was reduced towards the end of the light period when the animals approached a more starved state. Unfortunately Badawy *et al.* (1983) have given no information on when their experiments were performed in relation to the consumption of food or the onset of the light period. Furthermore it is not indicated how the fed state was ensured. It is therefore uncertain whether differences in feeding regimens could explain the discrepancy between their results and ours.

When continuous observations are made from an individual homogenate preparation, we consistently observe a decrease of absorbance in the absence of tryptophan, and have corrected for this 'blank' value. This apparently small, but, however, significant decrease may not be observed if results are pooled from several rat preparations. It is unclear whether this had been done by Badawy *et al.* (1983). These authors also appear to have

measured the enzyme activity without taking into account the lag phase which they have previously reported (Badawy & Evans, 1973, 1975).

The data underlying the study of Badawy *et al.* (1983) give the impression that the assay employed demonstrates great variability. We confirmed this when we tried to repeat the study using their method for the enzyme assay as described earlier (Badawy & Evans, 1973, 1975). We found that this variation was due to inadequate neutralization of some assay mixtures when trichloroacetic acid was used as a deproteinizing agent. Trichloroacetic acid has little or no buffer capacity and small errors in pipetting trichloroacetic acid caused large differences in pH values for replicate tubes. This caused variation in the absorbance readings if each tube was not individually titrated to neutrality with pH meter control. This is not the standard procedure in the assay as performed by Badawy *et al.* (1983), and is very time-consuming. We found that the variation could be reduced by replacing trichloroacetic acid with metaphosphoric acid (Stowell & Mørland, 1983) which has substantial buffer capacity at the desired endpoint pH. This allowed reproducible titration to be performed with a standard amount of NaOH for all samples run in the same incubation. Using this modified version of the method of Badawy *et al.* (1983), we found the blank reaction to be present in four consecutive experiments performed on fed rats. If not corrected for, this reaction would have influenced the enzyme activity reading markedly, for holoenzyme activities by as much as 50 per cent.

Badawy *et al.* (1983) have reproduced our finding that blood, remaining in the liver when it was frozen for later analysis, could increase haem availability to tryptophan oxygenase and thus lead to spuriously high holoenzyme activities in the subsequent assay. When they tested this without freezing the liver (a procedure we have not studied), they found no such increase. They concluded therefore that saline perfusion of the liver was not necessary when performing the enzyme assay on fresh tissue. We cannot disagree with this finding. Often, however, when performing time studies or investigating large series of animals, freezing of samples seems inevitable. Under these circumstances both Badawy *et al.* (1983), and ourselves would recommend perfusion of liver as described in our studies (Stowell &

Mørland, 1983) to avoid saturation *in vitro* of tryptophan oxygenase by haem derived from erythrocyte haemoglobin.

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