## A NOTE ON THE ESTIMATION OF TRIMETHYLAMINE IN FISH MUSCLE

The chemical methods for estimation of trimethylamine (TMA) in fish muscle consist of: 1) making a protein-free extract of tissue, 2) treating with formaldehyde (FA) to fix ammonia and amines other than tertiary amines, 3) volatilizing TMA with alkali into an organic phase (toluene), and 4) measuring the degree of ionization of picric acid in toluene caused by the extracted amine.

The methods have caused controversy; they have varied in detail such that the results from different laboratories have not always been in agreement (Shewan et al. 1971).

Bullard and Collins (1980) have recently compared methods of analysis for TMA and the interference by ammonia and dimethylamine (DMA). They included the method of Murray and Gibson (1972a) and confirm that 45% KOH as alkali is optimal for the release of TMA into the organic phase. They showed that the recovery of DMA is higher than is acceptable and that DMA interferes with the assays for TMA. Murray and Gibson (1972b) had found that the interference was very low and insignificant.

Tozawa et al. (1971) showed that the concentration of FA was critical in order to minimize the interference from DMA (see their fig. 3). They found that 0.5-1.0 ml FA was optimal for a 4 ml sample and added the FA before the toluene. If less were added, significant interference from DMA occurred even with KOH as alkali rather than  $K_2CO_3$ .

Bullard and Collins (1980) added only 1 ml of 3.7% FA to the sample (4 ml) after the addition of toluene. FA and aqueous FA, which exists as methylene glycol, are soluble in toluene and thus the effective concentration of FA in the aqueous sample is probably much lower than the minimum recommended by Tozawa et al. (1971) and is in the range which would cause maximum interference by DMA.

Murray and Gibson (1972a) used 1 ml of 50% neutralized FA added before toluene. They compared their procedure with that of Tozawa et al. (1971) and found no significant differences (unpubl. results). In addition they examined chromatographically the toluene phase after extraction and found that in their procedure only TMA was extracted (Murray and Gibson 1972b).

Thus it would have been more realistic and fair to previous workers if Bullard and Collins (1980)

had compared the actual published methods rather than their modifications to them and if they had specifically analyzed the material extracted into the toluene fraction. Accordingly their claim to have improved the method for TMA analysis cannot be substantiated.

It would be interesting to compare the results of analysis of samples from different species using the actual published methods done by an independent laboratory.

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D. M. GIBSON

Torry Research Station 135 Abbey Road Aberdeen AB9 8DG Scotland