A Modification of the Butanol Extraction Method for the Fluorimetric Assay of Catecholamines in Biological Materials

SIR,—A satisfactory and widely used method for the extraction of catecholamines from tissues is that described by Shore and Olin (1958). However, errors may arise due to the destruction of the amines during homogenisation and during the extraction period. The use of a motor-driven glass homogeniser may produce severe local over-heating even when the rotor is filled with ice.

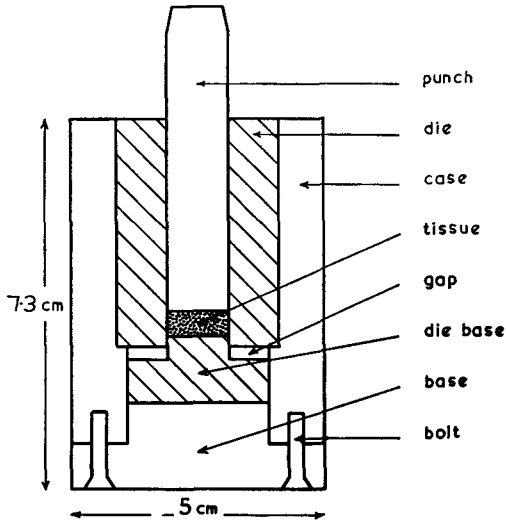

FIG. 1. A longitudinal section of the tabletting die and punch. The punch and die base are made of tool steel and the remainder is made of stainless steel. The die and die base are removable. A die of internal diameter of 1·2 cm. is suitable for the hearts of rats of 200 g. or less. For rat brains and hearts of animals of more than 200 g. a 2·0 cm. die is used.

This is particularly so with tough tissues such as heart and skin. Errors may also be produced by the presence of froth in some extracts, especially those from brain. This causes variations in the amounts of tissue in the samples when measured by pipette. All these factors lead to losses of the catecholamines and low assay results. A modification of the method which overcomes these difficulties has been developed.

The tissue to be assayed is placed in a stainless steel mesh basket and immersed in liquid nitrogen contained in a wide mouthed vacuum jar. When the nitrogen stops boiling the tissue is removed and dropped whole into a suitably sized stainless steel tablet die (see Fig. 1). The frozen tissue is crushed by striking the punch with a hammer. The entire compressed pellet of still-frozen tissue is expelled into the salt saturated butanol which is used in the Shore and Olin method. A volume of 0·01N HCl sufficient to produce a total aqueous phase of approximately 1/10th of the volume of butanol is added, together with 10 mg. of sodium metabisulphite to reduce oxidation during the extraction process. The remainder of the assay procedure is the same as that described by Cass and

Spriggs (1961). A comparison of the assay results obtained following homogenisation and following freezing in liquid nitrogen is shown in Table I.

TABLE I

A COMPARISON OF THE VALUES FOR THE NORADRENALINE CONTENTS (IN μG./G. OF TISSUE) OF RAT HEARTS AND BRAINS FOLLOWING (I) ACID HOMOGENISATION AND (II) FREEZING IN LIQUID NITROGEN

Tissue	(I) Acid homogenised			(II) Nitrogen extracted		
	No. of values	Mean content	Standard error	No. of values	Mean content	Standard error
Heart	15	0·80	±0·08	20	1·37	±0·14
Brain	18	0·35	±0·03	20	0·43	±0·02

It can be seen from the Table that the method described above yields values for the noradrenaline contents of rat hearts and brains that are significantly higher (brains: $P = 0{\cdot}05$, hearts: $P = 0{\cdot}01$) than those obtained following homogenisation. The sensitivity of the assay method is also improved since the values of the tissue blanks were reduced by 50 per cent. The greater difference in values for the rat hearts is due to the fact that the heart is a very tough tissue to homogenise and a great deal of local heating probably occurs whereas the brain is relatively soft and readily breaks down. The use of the entire pellet of tissue removes the difficulty caused by frothy extracts and further increases the sensitivity of the method.

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