INTRODUCTION

Much attention has been focussed in recent years on the metabolic transformation of glutamate, for which brain tissue is better equipped enzymatically than any other tissue of the body. From glutamate, \( \gamma \)-aminobutyric acid (GABA) is formed by the action of glutamate decarboxylase. Moreover, \( \gamma \)-aminobutyric acid which has been shown to be identical with factor I (2 and 6) has attracted much attention because of its inhibitory effect on synaptic transmission. Accordingly numerous studies have been done on the distribution and physiological action of these substances in brain tissue. Relatively detailed accounts of the regional distribution of \( \gamma \)-aminobutyric acid in the brains of rat (1, 4) and monkey (7 and 8) have been given as well as microchemical data on the concentration in layers of cerebral and cerebellar cortex (3) are also available. But in all such studies, the estimation process of GABA involved spectrophotometry, spectrofluorophotometry, radio isotope, paper chromatography, etc. None of the estimation was made by thin-layer chromatography. The present study has been carried out in order to establish the thin-layer chromatographic (TLC) procedure for the assay of GABA from the rat brain. The assay involves essentially the same principle as it is applied for other TLC methods and the detailed procedure is described in this communication.

MATERIALS AND METHODS

**Extraction method:**

Rats were killed by decapitation and brains were immediately removed and weighed, placed into small vials containing 4 to 6 ml of ice-cold 80% ethanol. Brain was homogenised...
in ice-cold 80% ethanol to obtain 10% suspension and was transferred quantitatively into a centrifuge tube. The homogenate was spun at 2000 g for 15 min. The supernatant was decanted. The precipitate was re-extracted twice with 3 ml of 80% ethanol and pooled extracts were evaporated to dryness under infrared lamp with the aid of a fan. One ml tripple glass distilled water was added to dried extract for every 100 mg of original fresh weight of the tissue. The extract was triturated thoroughly and the suspension was spun in a cold centrifuge at 20,000 g for 30 min. Aliquots of the supernatant were pipetted out and were used for spotting on TLC plates.

**Thin Layer Chromatographic Method (TLC):**

Various quantities of aliquots were spotted on activated TLC plates quoted with silica gel G having the thickness of 0.5 mm. Water saturated phenol was used as solvent system. After the run, the plates were kept in a fuming chamber for 6-8 hr for thorough removal of the phenol. This was tested qualitatively with F.C. reagent. The plates were then sprayed with 0.1 per cent solution of ninhydrin in butanol and the colour development was allowed to take place for 24 hr in the dark at room temperature. This procedure gave satisfactory result for the development of colour and differed from other amino acids which developed the colour completely only at higher temperature. Latter procedure did not yield satisfactory result with GABA. The developed spots were scraped out, care being taken to include same total area of the gel in all the known and unknown samples in any set of determinations. Suitably chosen silica gel blanks from the same plate were always included.

The scraped silica gel was put into test tubes and 5 ml of distilled water was then added. Tubes were shaken vigorously, to achieve quantitative elution of the colour. The silica gel was removed by centrifugation and the colour was read at 570 nm in a Carl Zeiss Spectrophotometer.

**RESULTS AND DISCUSSION**

**Recovery :**

Table I shows the recovery of GABA. Recoveries of 93 to 98 percent relative to the internal standard were obtained on addition of extracts from the brain.

**TABLE I : Percent recoveries of GABA.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration of GABA (µg)</th>
<th>O.D. (E₅₇₀)</th>
<th>O.D. of brain aliquot (µl) (E₅₇₀)</th>
<th>O.D. of I+II (E₅₇₀)</th>
<th>Percentage of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.060</td>
<td>0.030</td>
<td>0.088</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>3.00</td>
<td>0.120</td>
<td>0.030</td>
<td>0.147</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>6.00</td>
<td>0.240</td>
<td>0.030</td>
<td>0.262</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>9.00</td>
<td>0.360</td>
<td>0.030</td>
<td>0.385</td>
<td>98</td>
</tr>
</tbody>
</table>

Sensitivity:  
B: to be as  
Precision:  
Reproducibility of variation was 221 to 225 with the 240 µg/g. 
Specificity:  
Not present in other amino acids, this method is as low as
Estimation of GABA by TLC

A calibration curve for GABA shows linearity over the range of 1.5 µg to 9 µg (Fig. 1).

Fig. 1: Concentrations of GABA and optical density.

Sensitivity:
By suitable dilution of a standard solution, the minimum detectable level was found to be as low as 0.5 µg.

Precision:
Replicate analysis of GABA extracted from the rat brains showed 1.13% as coefficient of variation. It has been reported that in adult rat brain, GABA concentrations varied from 221 to 263 µg/g tissue (5). Results obtained in the present investigation agree quite well with these levels. The range of GABA concentration in the present experiment was 231 to 240 µg/g tissue with mean value 236.22 µg/g tissue (S.D. ± 2.67) while coefficient of variation was only 1.31 percent.

Specificity:
No interference was observed from other common amino acids which are generally present in the adult rat brain. The major advantage of this TLC method over the available paper-chromatography method, is the rapidity of estimation from the brain. Furthermore, this method is very much sensitive, could detect the presence of GABA in a concentration as low as 0.5 µg.
REFERENCES