cholamines promote REM sleep[4]; our data indicate that in man catecholamines tend to inhibit and serotonin tends to enhance REM sleep.

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Synthesis of γ-Aminobutyric Acid in Fish Erythrocytes

WE have found evidence of high activity of the enzyme glutamate decarboxylase (GAD) outside the nervous system. This enzyme catalyses the formation of γ-aminobutyric acid (GABA), which is believed to be an inhibitory neurotransmitter[1]. Recently, however, a high concentration of GABA (11 mmoles/kg wet weight) was found in flounder (*Pleuronectes flesus* L.) erythrocytes, where GABA may participate in the maintenance of a steady cell volume during changes in plasma osmolality[2,3].

The formation of GABA from glutamate was demonstrated as follows. Erythrocytes were homogenized in isotonic saline and incubated for 1 h at 10° C with 0·6 μmole/ml. homogenate of DL-[5-^{14}C]glutamate (specific activity 3·7 mCi/mmole, New England Nuclear Corp.). Amino-acids were extracted with alcohol and separated by thin-layer chromatography[3]. Spots were visualized by autoradiography and by treatment with ninhydrin reagent. The main labelled ninhydrin-positive product could not be distinguished from GABA (Fig. 1, *a*5 and *b*5). Radioactivity was also found in three other substances, one of which (Fig. 1, *b*6) was tentatively identified as α-ketoglutarate. No metabolism of GABA was detected by incubating with 5-^{14}C-GABA in similar conditions.

GAD activity was measured at 25° C by a micro method[4] depending on the production of $^{14}CO_2$ from L-[1-^{14}C]-glutamate (California Biochemical Corp.). The incubation mixture contained (final concentration): 3·5 mM sodium L-glutamate (75,000 c.p.m.), 20 mM sodium phosphate buffer (*p*H 6·5), 1 mM dithiothreitol, 0·1 mM pyridoxal phosphate and 0·25 per cent of 'Triton X-100'. In these conditions the activity of rat brain was 4·70 ± 0·25

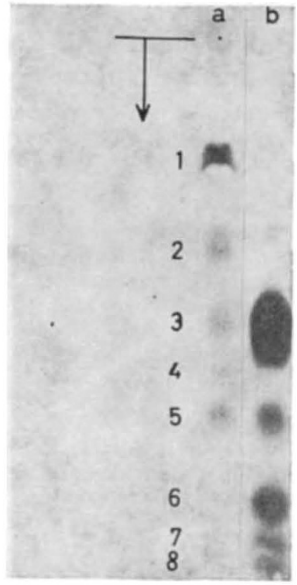

Fig. 1. *a*, Chromatogram of alcohol extract from flounder erythrocytes, showing the ninhydrin-positive spots (1–5); 3 = glutamate; 5 = GABA. *b*, Autoradiogram of the chromatogram *a*, showing the radioactive spots (3 and 5–8); 3 = glutamate; 5 = GABA; 6 = α-ketoglutarate.

μmoles/h/g wet weight (mean ± s.e., three experiments). In flounder, the following GAD activities (μmoles/h/g wet weight) were found: erythrocytes, 0·93 ± 0·08 (mean ± s.e., six animals); brain, 2·65 ± 0·27 (mean ± s.e., six animals). Other tissues contained low activities (liver, 0·06; kidney, 0·03—serum not detectable, one animal) some of which is accounted for by the presence of retained blood cells.

Like GAD of rat brain, the enzymes from flounder erythrocytes and brain were extractable with water, and were inhibited by high concentrations of Cl^- and by inactivation of pyridoxal phosphate. Thus, the erythrocyte enzyme was inhibited 77 per cent by 300 mM KCl and 69 per cent by 2 mM NaCN. The figures for the brain enzyme were 88 per cent and 72 per cent. K_m values measured at 25° C were for flounder erythrocytes 2·3 mM, for flounder brain 24 mM and for rat brain 12 mM (means of two experiments). It is interesting that flounder erythrocytes contain a low concentration of glutamate (less than 0·2 mmole/kg wet weight)[3].

GAD had been thought to occur solely in nervous tissue[1], but has recently been observed in mammalian kidney[5,6]. The kidney enzyme, however, differs from GAD of brain and of flounder erythrocytes for it is activated by Cl^- and probably does not require pyridoxal phosphate[5].

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