Rodrigues, V. and E. Buchner. Max-Planck-Institut für biologische Kybernetik, Tübingen, FR Germany.
Choline uptake in **Drosophila melanogaster** is linked to acetylcholine synthesis.

Radioactive choline, when injected into the haemolymph of living flies accumulates in specific regions of the brain (Buchner & Rodrigues 1983). The uptake is energy dependent and sensitive to the choline analogue, hemicholinium-3. The metabolism of acetylcholine following uptake of choline has been examined in **Drosophila** larval brains (Wu et al. 1983). Such studies in adult brains have proved difficult because of the high activity here of the acetylcholine hydrolytic enzyme, acetylcholinesterase. Much of the enzyme is present in a form which is easily rendered soluble, leading to the breakdown of acetylcholine during extraction procedures. We have overcome this problem by the use of tetraisopropyl-pyrophosphoramide (iso-OMPA) which is about 1000 fold more effective at inhibiting cholinesterases than eserine sulfate (Zingde et al. 1983).

20 µCi [methyl-^{3}H] Choline chloride (Sp. Act 15 Ci/mmol; Amersham) was injected into the haemolymph of female **Drosophila.** After incubation, under various conditions, at room temperature, the brains were rapidly dissected out and each placed in 15 µl extraction buffer (0.47M formic acid; 1.4M acetic acid; 10 mM iso-OMPA). The samples were freeze-thawed (6 times) and homogenized. The homogenate was spun at 15000g for 15 mins and the supernate lyophilized to 1 µl. Samples were spotted on a HPTLC cellulose plate (Merck 5787) and developed in a n-butanol: acetic acid: water: ethanol system (16:2:6:4). Radioactive spots were visualised by autoradiography on [^{3}H]-sensitive film (LKB, Sweden), (Fig. 1).

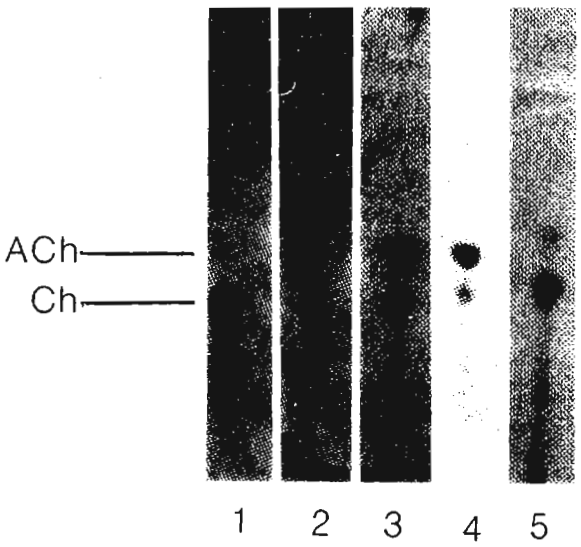

Figure 1. Detection of acetylcholine synthesis after [^{3}H] choline uptake in the brain of **Drosophila melanogaster.**

[^{3}H] Choline chloride (lane 1) and [^{3}H] acetylcholine chloride (lane 2) were run alongside as standards. After 30 mins of incubation the radioactivity was distributed approximately equally between choline and acetylcholine (lane 3). This includes the label present in both the intracellular and extracellular compartments of the brain. Incorporation of label into choline containing lipids was not observed in our experiments. Longer incubation times led to an increased conversion of choline to acetylcholine (60 mins in lane 4).

The extract in lane 5 is from a fly which was injected with 2nMoles of hemicholinum chloride together with [^{3}H] choline. The synthesis of acetylcholine is markedly inhibited. The radioactive spot could represent the choline in the extra-cellular spaces since previous studies have shown that hemicholinium-3 inhibits the specific uptake of choline.

References: Buchner, E. & V. Rodrigues 1983, Neuroscience Letters 42: 25-31; Wu, C.E. et al. 1983, J. Neurochem. 40: 1386-1396; Zingde, S. et al. 1983, J. Neurochem. 41: 1243-1252.