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## The effect of fractionation conditions on subcellular distribution in brain

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WHITTAKER and Dowe[1] have shown that the yield of acetylcholine (ACh) in a brain homogenate is dependent upon the shearing forces applied. However, their objection to the Emanuel-Chaikoff piston-press homogenizer, used by Ryall,[2] is not valid on the evidence which they presented. The main part of their argument was based upon one experiment extracted from a paper by Ryall,[2] and this has been misinterpreted. Whittaker and Dowe[1] calculated that the ACh content of a brain tissue homogenate obtained by Ryall[2] with the Emanuel–Chaikoff homogenizer was 4·7

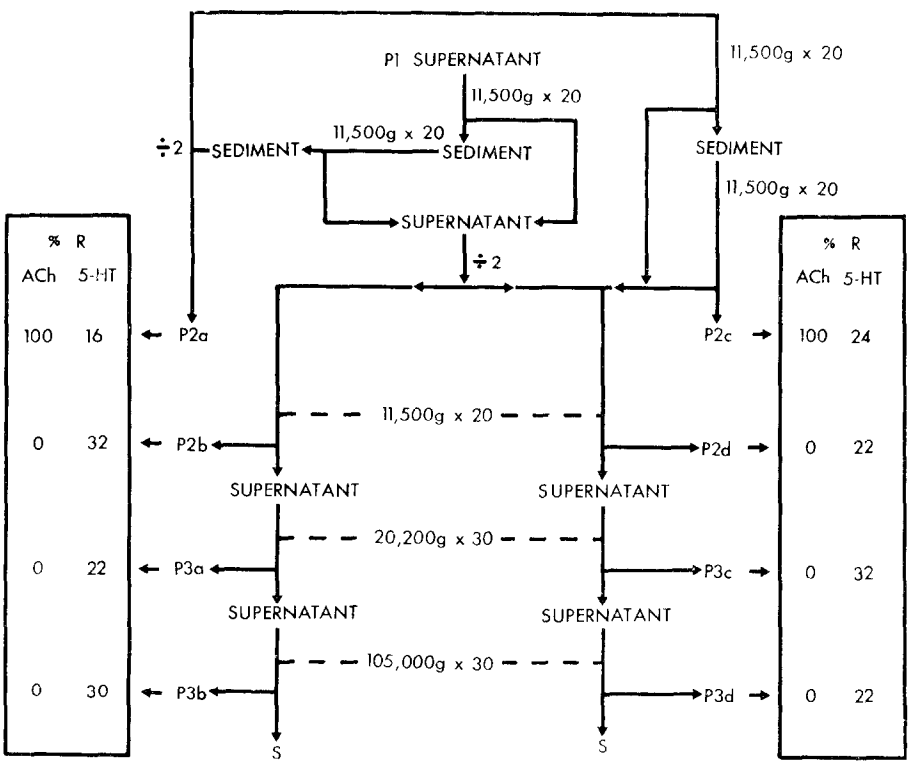


FIG. 1. Subcellular distribution of ACh and 5-HT in rat brain homogenates. The P1 supernatant was obtained as in an earlier investigation.[2] % R is the percentage of the total amount recovered in P2a, P2b, P3a and P3b or in P2c, P2d, P3c and P3d. ÷ 2 shows where the sediment or supernatant were divided into two equal portions. Centrifugal fields are shown as g × min. The results are the averages obtained in two experiments. The arrows indicate consecutive steps in the separation.

mμM/g. This figure is slightly incorrect; (a) the ACh recovery was 0·85μg of base (i.e. of cation) per gramme of tissue, i.e. 5·8 mμM/g, (b) this was the amount recovered in P1, P2 and P3, and not in the homogenate as shown by Whittaker and Dowe,[2] and about 10 per cent of the ACh was lost during the separation of these fractions (see also Ref. 1). A more serious mistake was their failure to observe that, although the value in this particular experiment was low (probably due to different depths of anaesthesia and speed of removal of the brain[2, 3] which could be a major cause of discrepancies between different laboratories), it was not representative and there was no significant difference between the *average* amounts of 'bound' ACh (i.e. in P1, P2 and P3) in the presence or

absence of physostigmine (see Ref. 2 page 134). The only significant effect of physostigmine was to increase the amount of ACh in the supernatant fraction (see also Ref. 4). In fact, the *average* recovery of 'bound' ACh was 1·6 μg/g (11 mμM/g) in fractions pepared in media containing physostigmine (see Ryall[2], column 1, Table 1). This value is very similar to the value of 12·8 mμM/g quoted by Whittaker,[1, 3] and is also similar to the amount recovered by De Robertis, Pellegrino de Iraldi, Rodriguez de Lores Araniz and Salganicoff,[5] who used a glass and Teflon homogenizer. Thus it is re-emphasized that, insofar as comparisons between different laboratories are valid, there is no evidence to support the contention[1] that the preparation of a homogenate in a piston-press type of homogenizer under the conditions used by Ryall[2] resulted in a lower yield of 'synaptosomes', as indicated by the recovery of 'bound' ACh, than in the experiments of Whittaker and Dowe.[1]

Ryall[2, 6] reported results for the distribution of 5-hydroxytryptamine (5-HT) which differed from those obtained by Whittaker and his colleagues.[4, 7] Results similar to those obtained by Ryall[2, 6] with the Emanuel–Chaikoff homogenizer have been reported by two other independent groups of investigators,[8] who used glass and Teflon homogenizers. Thus, the different results for 5-HT cannot be attributed to the use of the Emanuel–Chaikoff homogenizer.

Ryall[2] suggested that it may ultimately be possible to completely separate the ACh and 5-HT containing particles in brain homogenates. The following results show that it was possible to effect an almost complete separation by differential centrifugation. They also indicated that the different results obtained by Michaelson and Whittaker[7] may have been due to the use of high gravitational fields for the separation of their P2 fraction, in contrast to the smaller gravitational fields used by others.[2, 6, 8]

The technique and results are summarized in Fig. 1. The results on the left side of the figure, obtained after only two centrifugations and one resuspension of the supernatant fluid remaining after removal of the 'nuclear' (P1) fraction, were not very different from those obtained after four centrifugations and three resuspensions (right side of Fig. 1). Two sedimentations at 11,500 g for 20 min were sufficient to remove all the ACh-containing particles but only about 16 per cent of the 5-HT. The remainder of the 5-HT-containing particles were sedimented in each of the subsequent centrifugations. It will be noted that the amount of 5-HT remaining after sedimentation at 20,200 g for 30 min (606,000 g min) was only 20–30 per cent of the total. It is therefore relevant that Michaelson and Whittaker[7] sedimented their P2 fraction at 17,000 g for 60 min (820,000 g min) and nearly all of the particle-bound 5-HT was found in their P2 fraction, together with ACh.

These results support the previous conclusion[2] that the bound 5-HT is located in particles distinct from those which contain ACh, and that the former particles may be of microsomal dimensions, although they are apparently not synaptic vesicles.[8, 9] This suggestion may explain why 5-HT has been located histochemically[10] in both axons and cell bodies, as well as in terminals.

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