the tissue half-life of bound histamine was in excess of 50 days; consequently, prolonged administration of a potential histidine decarboxylase inhibitor would be necessary to deplete tissue histamine stores. We attempted to anticipate this problem by giving large amounts of drug for several weeks. Failure to detect any depletion of tissue histamine in this study makes it unlikely that longer treatment with higher doses of these drugs would significantly change the results.

Me5HTP was rapidly absorbed and excreted; it appeared in the urine 30 min after i.p. administration and was completely absent 18 hr later. Since the drug was in the DL form, there might have been rapid excretion of nonmetabolized *dextro*-form. Nevertheless, the large amount excreted and the paucity of metabolites suggested that the *levo*-form was also little metabolized. Two other indoles appeared in the urine after Me5HTP administration. We suspect that one was α-methyl-5-hydroxytryptamine, the expected product of Me5HTP decarboxylation.

Acknowledgement—We wish to thank Mrs. Helen Vincent and Mr. Sam Fanous for their excellent technical assistance.

Department of Medicine,
University of California Center
for the Health Sciences,
Los Angeles, Calif., U.S.A.

GERALD R. MARSCHKE
GILDON N. BEALL

REFERENCES

1. C. F. CODE, *Amer. J. Physiol.* **127**, 78 (1939).
2. G. B. LOGAN, *Proc. Soc. exp. biol. (N.Y.)* **107**, 466 (1961).
3. W. SCHÜMUTZLER, H. GIERTZ and F. HAHN, *Int. Arch. Allergy* **22**, 69 (1963).
4. K. F. AUSTEN and W. E. BROCKLEHURST, *J. exp. Med.* **113**, 521 (1961).
5. A. L. MICHELSON and F. C. LOWELL, *New Engl. J. Med.* **258**, 994 (1958).
6. R. W. SCHAYER, *J. biol. Chem.* **199**, 245 (1952).
7. B. ROBINSON and D. M. SHEPHERD, *J. Pharm. Pharmacol.* **14**, 9 (1962).
8. P. A. SHORE, A. BURKHALTER and V. H. COHN, Jr., *J. Pharmacol. exp. Ther.* **127**, 182 (1959).
9. I. SMITH, *Chromatographic and Electrophoretic Techniques*, vol. 1, *Chromatography*, Interscience, New York (1960).
10. W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **237**, 89 (1962).

Biochemical Pharmacology, 1965, Vol. 14, pp. 194–196. Pergamon Press Ltd., Printed in Great Britain.

The effect of homogenization conditions on sub-cellular distribution in brain

(*Received* 5 *June* 1964; *accepted* 26 *June* 1964)

THE purpose of this communication is to draw attention to the effect which relatively small changes in homogenization conditions may have on the distribution of bound acetylcholine, and therefore possibly of other components in subcellular fractions derived from brain tissue.

When brain tissue is homogenized in eserine-free 0·32 M sucrose according to our usual procedure,[1,2] about 70–75% of the total acetylcholine of the tissue survives the action of the powerful cholinesterases present in the preparation and is recovered mainly in the crude mitochondrial (P_2) fraction and in a subfraction (B) derived from it, consisting largely of pinched-off presynaptic nerve terminals (synaptosomes). The acetylcholine content of brain fractions prepared in the absence of cholinesterase inhibitors such as eserine may therefore be used as a measure of the survival in the fractions of these organized neuronal elements.

In the present series of experiments, a comparison was made of the effect of three different methods of homogenization on the acetylcholine content of the homogenates and of the subcellular fractions derived from them by our standard procedure. The homogenates were prepared (i) mechanically, (a) according to our usual procedure using a glass and Perspex homogenizer of the type described by Aldridge, Emery and Street,[3] with a clearance of 0·25 mm, a speed of rotation of the pestle of 840 rev/min and twelve up-and-down motions of the mortar during preparation (Table 1, column 1),

TABLE 1. EFFECT OF HOMOGENIZATION CONDITIONS ON THE YIELD OF SYNAPTOSOMES FROM WHOLE GUINEA PIG AND RAT BRAIN

Type of homogenizer	Aldridge		Modified Dounce	Emanuel-Chaikoff*
Species	guinea pig		guinea pig	rat
Clearance (mm)	0·25		0·12	0·027
Speed (rev/min)	840	1400	—	—
Acetylcholine in homogenate (mμmoles/g tissue)	12·8	7·0	11·9	4·7
Distribution of acetylcholine in fractions (% of recovered activity)				
P_1	12	8	35	5
P_2	70	83	46	82
S_2	18	9	19	13
Recovery (% of homogenate)	100	94	93	—

* Calculated from results of Ryall.[4] Rat brain homogenates prepared as in column 1 have an acetylcholine content similar to that of guinea pig brain homogenates prepared in the same way.

(b) in the same way except that the speed of rotation was raised to 1400 rev/min (column 2) and (ii) by hand, using a modified Dounce-type homogenizer (column 3). This consisted of a flat-bottomed conical stainless steel pestle moving in a flat-bottomed, smooth-bore glass mortar, with a clearance between the base of the conical pestle and the walls of the mortar of 0·12 mm. Three fractions were prepared from each homogenate, a crude nuclear (P_1) fraction, sedimented at 1000 *g* for 11 min and washed twice by resuspension in sucrose and resedimentation, a crude mitochondrial fraction (P_2) sedimented from the combined supernatants from fraction P_1 at 17300 *g* for 55 min and the remaining supernatant (S_2). Some results of Ryall,[4] using an Emanuel-Chaikoff homogenizer and a similar procedure for preparing the fractions, are inserted for comparison (column 4).

It will be seen that the yield of synaptosomes as indicated by the acetylcholine content of the initial homogenate and of the P_2 fraction falls when the rate of shear in the Aldridge homogenizer is increased (column 2); it falls still further with the very disruptive type of homogenization produced by the piston-press homogenizer (column 4). By contrast, the milder conditions prevailing during hand-homogenization (column 3) do not greatly affect the amount of acetylcholine in the initial homogenate, but the proportion recovered in fraction P_1, representing incompletely homogenized tissue fragments, is considerably higher, and the yield of synaptosomes again lower than with the Aldridge homogenizer using a moderate rate of shear. It would thus appear that the conditions originally selected in our work are nearly optimal for the formation of synaptosomes. Variations in the yield of synaptosomes with different homogenization techniques and media[2] may well explain some of the differences being reported in the literature for the subcellular distribution of biogenic amines and enzymes in brain fractions. In particular, the use of the Emanuel-Chaikoff homogenizer seems inadvisable in this kind of work. Careful standardization of conditions will clearly be necessary before valid comparisons can be made between the results from different laboratories.

Acknowledgement—This investigation was supported by a grant No. NB 03928-02 (to V.P.W.) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, U.S. Public Health Service.

Biochemistry Department,
Agricultural Research Council,
Institute of Animal Physiology,
Babraham, Cambridge, England

V. P. WHITTAKER
G. H. C. DOWE

REFERENCES

1. V. P. WHITTAKER, *Biochem. J.* **72**, 694 (1959).
2. E. G. GRAY and V. P. WHITTAKER, *J. Anat.*, (*Lond.*), **96**, 79 (1962).
3. W. N. ALDRIDGE, R. C. EMERY and B. W. STREET, *Biochem. J.* **77**, 326 (1960).
4. R. W. RYALL, *J. Neurochem.* **11**, 131 (1964).