- Bernheim, F. & Bernheim, M. L. C. (1933) *Amer. J. Physiol.* **104**, 438–440
- Besson, M. J., Cheramy, A., Feltz, P. & Glowinsky, J. (1969) *Proc. Nat. Acad. Sci. U.S.A.* **62**, 741–748
- Chakrin, L. W. & Whittaker, V. P. (1969) *Biochem. J.* **113**, 97–107
- Chakrin, L. W., Marchbanks, R. M., Mitchell, J. F. & Whittaker, V. P. (1972) *J. Neurochem.* **19**, 2727–2736
- Collier, B. (1969) *J. Physiol. (London)* **205**, 341–352
- Diamond, I. & Kennedy, E. P. (1969) *J. Biol. Chem.* **244**, 3258–3263
- Dowdall, M. J., Barker, L. A. & Whittaker, V. P. (1972) *Biochem. J.* **130**, 1081–1094
- Fonnum, F. (1967) *Biochem. J.* **103**, 262–270
- Fonnum, F. (1969) *Biochem. J.* **113**, 291–298
- Furshpan, E. J. (1956) *J. Physiol. (London)* **134**, 689–697
- Gradijan, J. R. & Bergner, P.-E. E. (1972a) *Biometrics* **28**, 313–328
- Gradijan, J. R. & Bergner, P.-E. E. (1972b) *Biochem. J.* **130**, 1075–1080
- Gray, E. G. & Whittaker, V. P. (1962) *J. Anat.* **96**, 79–88
- Hebb, C. O. & Whittaker, V. P. (1958) *J. Physiol. (London)* **142**, 187–196
- Hubbard, J. I. & Kwanbunbumpen, S. (1968) *J. Physiol. (London)* **194**, 407–420
- Jones, S. F. & Kwanbunbumpen, S. (1970) *J. Physiol. (London)* **207**, 31–50
- Kopin, I. J., Bresse, G. R., Krauss, K. R. & Weise, V. K. (1968) *J. Pharmacol. Exp. Ther.* **161**, 271–278
- Liang, C. C. & Quastel, J. H. (1969) *Biochem. Pharmacol.* **18**, 1187–1194
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Luparello, T. J. (1967) *Stereotaxic Atlas of the Forebrain of the Guinea Pig*, Williams and Wilkins, Baltimore
- Mann, P. J. G. & Quastel, J. H. (1937) *Biochem. J.* **31**, 869–878
- Marchbanks, R. M. (1969) *Symp. Int. Soc. Cell Biol.* **8**, 115–135
- Marchbanks, R. M. & Israël, M. (1971) *J. Neurochem.* **18**, 439–448
- Potter, L. T. (1970) *J. Physiol. (London)* **206**, 145–166
- Richter, J. A. & Marchbanks, R. M. (1971) *J. Neurochem.* **18**, 705–712
- Schubert, J. & Sundwall, A. (1967) *J. Neurochem.* **14**, 807–812
- Schubert, J., Sparf, B. & Sundwall, A. (1969) *J. Neurochem.* **16**, 695–700
- Weakly, J. N. (1969) *J. Physiol. (London)* **204**, 63–77
- Whittaker, V. P. (1965) *Progr. Biophys. Mol. Biol.* **15**, 39–96
- Whittaker, V. P. (1969) *Progr. Brain Res.* **31**, 211–222
- Whittaker, V. P. (1971) *Abstr. Int. Meet. Int. Soc. Neurochem.* **3rd**, 441
- Whittaker, V. P. & Barker, L. A. (1971) in *Methods of Neurochemistry* (Fried, R., ed.), vol. 2, pp. 1–52, Marcel Dekker Inc., New York
- Whittaker, V. P., Michaelson, J. A. & Kirkland, R. J. A. (1964) *Biochem. J.* **90**, 293–303

APPENDIX

A Mathematical Model of the Acetylcholine Compartments in Synaptosomes

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(Received 24 August 1972)

To examine the time-course of labelling of the various compartments of bound acetylcholine in synaptosomes, the linear model shown diagrammatically in Scheme 1 was investigated. Radioactive choline, in concentration x_0 , is taken up into the synaptosome cytoplasm from the extracellular fluid at a rate defined by the rate constant l . It penetrates to the centre of the synaptosome, mixing with the endogenous pool *en route*, through a series of concentric spherical zones here represented as a linear series of n compartments running from the periphery to the centre of the synaptosome. The rate constant defining the rate of diffusion of choline from one cytoplasmic compartment to the next is k_2 . Meanwhile, choline (whether as such or as acetylcholine is immaterial for the purpose of the present analysis) is assumed to be taken up by vesicles in contact with the n compartments at a rate defined by the constant k_1 . Finally, radioactivity is being lost from the vesicles at a rate defined by the constant k_3 . The concentra-

tions of radioactivity at any given time t , in the vesicles and in the regions of cytoplasm with which they are in contact are denoted by x_1 to x_n and x'_1 to x'_n respectively.

It is further assumed that vesicles can be divided into two classes: metabolically more active ('P-type') vesicles (1 to j) close to the periphery of the synaptosome, of the type mainly recovered in fraction H , and metabolically less active ('C-type') vesicles ($j+1$ to n) nearer to the centre of the synaptosome and of the type mainly recovered in fraction D . The concentrations of radioactivity x_1 to x_n present in these vesicles are defined by rate constants (for P-type vesicles) k_1^P and k_3^P and for C-type vesicles k_1^C and k_3^C , the assumption being made that $k_1^P > k_1^C$ and $k_3^P > k_3^C$.

Finally, as a further refinement of the model, it is assumed that the partitioning of vesicles between fraction H (vesicles 1 to y) and fraction D (vesicles $y+1$ to n) may vary from one experiment to another, so that y is not necessarily equal to j .

Scheme 1. Linear model of the compartments of acetylcholine in synaptosomes

For explanation of symbols, see the text.

The amounts of radioactivity at time t in fractions H and D , namely $h(t)$ and $d(t)$ respectively, are:

$$h(t) = \sum_1^y x_i \quad \text{and} \quad d(t) = \sum_{y+1}^n x_i$$

The values of x_i are obtained from the following set of differential equations, expressed in matrix form (eqn. 1):

Numerical solutions to this set of differential equations and hence numerical values for $h(t)$ and $d(t)$ were obtained by means of the Hamming Predictor-Corrector Method (Ralston & Wilf, 1960) by using an IBM 360 computer. To determine a set of parameter values that would give a reasonably good qualitative fit to the experimental data, a reduced version of the model was first made on an EAI 680 analogue computer.

$$\begin{bmatrix} \dot{x}_0 \\ \dot{x}_1 \\ \dot{x}_1' \\ \dot{x}_2 \\ \dot{x}_2' \\ \vdots \\ \dot{x}_{n-1} \\ \dot{x}_{n-1}' \\ \dot{x}_n \\ \dot{x}_n' \end{bmatrix} = \begin{bmatrix} -l & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 \\ 0 & -k_3^P & k_1^P & 0 & 0 & \cdots & 0 & 0 & 0 & 0 \\ l & 0 & -(k_1^P + k_2) & 0 & k_2 & \cdots & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_3^P & k_1^P & \cdots & 0 & 0 & 0 & 0 \\ 0 & 0 & k_2 & 0 & -(k_1^P + 2k_2) & \cdots & 0 & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & \cdots & -k_3^C & k_1^C & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \cdots & 0 & -(k_1^C + 2k_2) & 0 & k_2 \\ 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & -k_3^C & k_1^C \\ 0 & 0 & 0 & 0 & 0 & \cdots & 0 & k_2 & 0 & -(k_1^C + k_2) \end{bmatrix} \begin{bmatrix} x_0 \\ x_1 \\ x_1' \\ x_2 \\ x_2' \\ \vdots \\ x_{n-1} \\ x_{n-1}' \\ x_n \\ x_n' \end{bmatrix}$$

Fig. 1 shows the values of $h(t)$ and $d(t)$ at different times when $n = 20$, $j = y = 5$, $k_1^P = 0.2$, $k_1^C = 0.1$, $k_2 = 0.5$, $k_3^P = 2.0$, $k_3^C = 0.15$ and $l = 2.0$. The curves show some of the characteristics of the experimental results; however, although the peak in the $h(t)$ curve is larger in amplitude relative to the $d(t)$ curve, it occurs earlier; and the peak in the $d(t)$ curve does not have the somewhat flattened form ('plateau') seen in the experimental results.

Lowering the k_1^P/k_1^C ratio or raising the value of k_2 relative to the other constants caused the curves for $h(t)$ and $d(t)$ to approach each other in shape, size and position. Thus, to approximate to the experimental results it was necessary to assume (a) that P-type vesicles were metabolically more active than C-type vesicles, (b) that rapid mixing of incoming choline did not take place.

Effect of random variations in y

Since the experimental results represented the sampling of a large variety of synaptosomes, it was felt that better qualitative agreement between the model and the experimental findings might be obtained by simulating random sampling.

One possible source of randomness might be the inclusion of varying amounts of C-type vesicles in fraction H and vice versa. This type of randomness was simulated by keeping $j = 5$, varying y according to a prescribed distribution and calculating the mean and standard deviation of the resulting $h(t)$ and $d(t)$ curves. Fig. 2 shows the result of such a procedure in which y was given a uniform distribution over the range 1 to 5. This had the effect (Fig. 2) of flattening the peak in the $d(t)$ curve, but, in marked contrast to the experimental findings, the variance in the $h(t)$ and $d(t)$ curves was identical.

Effect of random variations in l

Another possible source of randomness arises from the fact that the nerve terminals are located at different distances from the injection site; the nearer the terminal is to the injection site, the more rapidly will uptake occur.

We have sought to incorporate this element of randomness into the model by assuming that all terminals receive a unit amount of tracer, and that l varies according to the following weighted distribution: 2.0 (3), 1.0 (3), 0.5 (2), 0.2 (1), 0.1 (1); where the values in parentheses are the weights assigned to the corresponding values of l (x_0 could have been varied instead of l , but this would make little difference to the results). It will be seen (Fig. 3) that the mean

curves are similar to the deterministic curves (Fig. 1) but that the standard deviations of points in the $h(t)$ curve are larger than those of points in the $d(t)$ curve.

Effect of variations in both y and l

Since variation in y produces the plateau in $d(t)$ and variations in l the difference in variance between $h(t)$ and $d(t)$, an attempt has been made to reproduce both features simultaneously by varying y and l together. The combined probability distribution for y and l was the product of the probability distributions used for y and l separately (that is, y and l are assumed to vary independently). Fig. 4 shows that the combined variation produces curves having the two features of a flattened peak in $d(t)$ and a greater variance in $h(t)$ than in $d(t)$.

Relation to specific radioactivity

It should be noted that $h(t)$ and $d(t)$ represent concentrations of tracer rather than specific radioactivity. To obtain the latter, the values for $h(t)$ and $d(t)$ must be divided by the steady-state solution of eqn. 1, i.e. the solution when $\dot{x}_0 = -1$ and all other derivatives are set equal to zero. This would have the effect of raising the $h(t)$ curve, lowering the $d(t)$ curve and pushing the cross-over point to a long time-interval and low specific radioactivity. A cross-over was not observed experimentally, probably because observations were not made at sufficiently long times after choline injections.

Discussion

In conclusion, it should be emphasized that the aim in studying this mathematical model of the acetylcholine compartments of the synaptosome has not been to obtain a good quantitative fit to the data but to see whether the model makes predictions qualitatively similar to the observed results. It is clear that to obtain qualitative agreement it is necessary to make the following minimal assumptions (a) that there are two types of vesicles (or osmotically stable storage sites) differing in metabolic activity, the metabolically more active being preferentially recovered in fraction H , (b) the diffusion of incoming choline throughout the cytoplasm is a relatively slow process compared with the uptake of radioactivity into the vesicles, (c) the site of penetration of choline from the injection site into synaptosomes varies for different synaptosomes, (d) there is variation in the composition of fractions H and D in

Fig. 1. *Deterministic solution of the model shown in Scheme 1*

Values used were as follows: l and y fixed at values of 2.0 and 5.0 respectively, $n = 20$, $j = 5.0$, $k_1^p = 0.2$, $k_1^c = 0.1$, $k_2 = 0.5$, $k_3^p = 2.0$, and $k_3^c = 0.15$.

Fig. 2. *Effect of random variations in y*

Values were the same as in the legend to Fig. 1 except that y was given a uniform distribution over the range 1 to 5 (i.e. y assumed values of 1, 2, 3, 4 and 5 with equal probability). Vertical bars represent standard deviations.

Fig. 3. *Effect of random variations in l*

Values were the same as in the legend to Fig. 1 except that l was varied according to the following weighted distribution: 2.0 (3), 1.0 (3), 0.5 (2), 0.2 (1), 0.1 (1); where the values in parentheses are the weights assigned to the corresponding values of l . Vertical bars represent standard deviations.

Fig. 4. *Effect of simultaneous random variations in y and l*

Values were the same as in the legend to Fig. 1 except that both y and l were varied simultaneously and independently in the fashions described in the legends to Figs. 2 and 3 respectively. Vertical bars represent standard deviations.

regard to the proportion of the two types of osmotically stable storage sites sedimenting into each fraction.

A fuller account of the methods used has been published separately (Gradijan & Bergner, 1972).

This work was supported by General Research Support Grant no. FR-05561 and Grant no. MH-17566 from the

National Institutes of Mental Health, U.S. Public Health Service, Bethesda, Md., U.S.A.

References

- Gradijan, J. R. & Bergner, P.-E. E. (1972) *Biometrics* **28**, 313-328
Ralston, A. & Wilf, H. S. (1960) *Mathematical Methods for Digital Computers*, Wiley, New York