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APPENDIX

Mathematical Approaches to the Evaluation of the Flux of γ -Aminobutyrate in Brain Tissue *in vitro*

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In the preceding paper (Balázs, Machiyama, Hammond, Julian & Richter, 1970) the flux of γ -aminobutyrate (GABA) was found, in guinea-pig brain-cortex slices incubated in glucose-saline medium, to represent about 10% of the total tricarboxylic acid cycle flux, as opposed to other estimates, which are as high as 40%. However, the latter value was deduced from experimental results by methods that

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made no allowance for the metabolic compartmentation of glutamate: a mathematical investigation was therefore undertaken to show that this omission necessarily leads to an overestimation of GABA flux. The magnitude of this overestimation was shown by computer simulation methods to be of such an order as to bring the corrected value into agreement with the lower value. Computer simulation methods were also used to evaluate the GABA flux from the experimental results presented by Balázs *et al.* (1970) and a value of $0.0315 \mu\text{mol/min per g wet wt.}$ was obtained. This value was also shown to be consistent, in the simulated system, with the experimentally observed time-courses for the radioactivity and quantity of aspartate. Since there is now evidence that GABA is itself a metabolically compartmented intermediate this possibility was considered mathematically, but it was found that in this case the assumption of compartmentation had little effect upon the value of GABA flux deduced on the basis of GABA homogeneity.

Estimates of GABA* flux in brain tissue, expressed as a percentage of total flux through the tricarboxylic acid cycle, vary in the literature from about 10% to 40% but the preceding paper shows that those experiments and interpretations that give rise to the higher flux estimates are open to criticism (Balázs, Machiyama, Hammond, Julian & Richter, 1970). In seeking further information as to the value of the GABA flux, a mathematical approach has been undertaken to establish the error in estimation of flux that arises in the interpretation of the set of experiments that gave the value (40%) reported by McKhann, Albers, Sokoloff, Mickelson & Tower (1960). In their study the compartmentation of glutamate (the immediate precursor of GABA) was ignored (see Albers, Koval, McKhann & Ricks, 1961) and the present approach derives the flux error consequent upon this omission. However, the mathematical treatment is also of wider interest since it has relevance to all metabolic experiments in which glutamate or its precursors are labelled.

In studies that employ labelled precursors of GABA, the paucity of quantitative information about glutamate compartmentation gives rise to correspondingly uncertain values for GABA flux and the experiments reported in the preceding paper (Balázs *et al.* 1970) represent an attempt to avoid this uncertainty by the direct use of $[1-^{14}\text{C}]\text{GABA}$ as the source of radioactive carbon. The experimental conditions, together with the assumption that GABA is not metabolically compartmented, allow the GABA flux to be readily deduced from the attrition of total GABA and $[1-^{14}\text{C}]\text{GABA}$. The value for the GABA flux so obtained may also be checked for consistency with the observed time-courses of radioactivity combined in metabolites associated with the tricarboxylic acid cycle.

During these studies, evidence was obtained that indicated metabolic compartmentation of GABA in brain tissue (Machiyama, Balázs, Hammond, Julian & Richter, 1970). Further mathematical studies were therefore undertaken to make a

quantitative assessment of the consequences that such compartmentation would have on the value of GABA flux previously evaluated on the basis of homogeneity.

METHODS

The biochemical reactions by which GABA is related to the tricarboxylic acid cycle in brain tissue are described in the preceding paper (Balázs *et al.* 1970) and lead to the simplified scheme shown in Scheme 1(a). The notation used in the figure defines the symbols that are used throughout the present paper and all equations are derived from Scheme 1(a) or, where compartmentation of glutamate or GABA are specifically considered, equations are derived from Scheme 1(b) or 1(c) respectively.

Method of assessment of errors due to glutamate compartmentation. In studies with labelled precursors of GABA, such as that of McKhann *et al.* (1960) with $[3-^{14}\text{C}]\text{pyruvate}$, the time-courses of the radioactivity contained in glutamate and GABA may be determined and, assuming an uncompartmented system, a value for GABA flux (K_u) may be deduced.

From the notation of Scheme 1(a):

$$K_u = C_1 S_1 = C_2 S_2$$

and

$$\dot{R}_2 = C_1 R_1 - C_2 R_2$$

$$\text{Therefore } \dot{R}_2/K_u = R_1/S_1 - R_2/S_2 \quad (1)$$

Since, however, glutamate is metabolically compartmented as in Scheme 1(b) the true GABA flux (K_c) is given by

$$K_c = C'_1 S'_1 + C''_1 S''_1 = C_2 S_2$$

where

$$\dot{R}_2 = C'_1 R'_1 + C''_1 R''_1 - C_2 R_2$$

Therefore

$$\dot{R}_2/K_c = [(C'_1 R'_1 + C''_1 R''_1)/(C'_1 S'_1 + C''_1 S''_1)] - R_2/S_2 \quad (2)$$

Eqns. (1) and (2) provide a means for comparing the two values of GABA flux, K_u and K_c , as deduced on the assumption of an uncompartmented and a compartmented system respectively:

$$K_c \leq K_u \text{ when } R_1/S_1 \leq (C'_1 R'_1 + C''_1 R''_1)/(C'_1 S'_1 + C''_1 S''_1)$$

* Abbreviation: GABA, γ -aminobutyrate.

Scheme 1. GABA systems as invoked for mathematical treatment. The quantities, radioactivities, utilization rate constants and utilization fluxes of substrates are represented by S , R , C and K respectively and are followed by suffixes that nominate the appropriate substrate. Systems involving metabolically compartmented glutamate and GABA are shown as (b) and (c) respectively, where the subpool parameters are indicated by the use of single and double primes. Other symbols are defined in the text.

This conditional statement may be simplified by using the identities $R_1 = R'_1 + R''_1$ and $S_1 = S'_1 + S''_1$ and then gives

$$K_c \leq K_u \text{ when } (C'_1 - C''_1)(a''_1 - a'_1) \leq 0 \quad (3)$$

where a'_1 and a''_1 are the specific radioactivities of their respective glutamate pools. Reference to Scheme 1(b)

Assuming that the two glutamate pools at zero time have constant contents of radioactivity (mathematically these need not be zero) then $a'_1(0) = a''_1(0)$, represented by $a_1(0)$. Under these conditions the solutions for a'_1 and a''_1 , of the form given by eqn. (4), may be substituted in the conditional statement eqn. (3) to give $K_c \leq K_u$ when

$$(C'_1 - C''_1)\{[a_1(0) - a_6(0)]\{\exp(-C'_1 t) - \exp(-C''_1 t)\} - \dot{a}_6[1/C'_1 - 1/C''_1] + \ddot{a}_6(0)[\exp(-C''_1 t)/C'_1 - \exp(-C'_1 t)/C''_1] + \ddot{a}_6[(1/C'_1)^2 - 1/(C''_1)^2] - \ddot{a}_6(0)[\exp(-C''_1 t)/(C'_1)^2 - \exp(-C'_1 t)/(C''_1)^2] - \dots\} \leq 0 \quad (5)$$

shows that these specific radioactivities are given by equations of the form

$$a'_1 = C'_1(a_6 - a'_1)$$

where a_6 is the specific radioactivity of the precursor of glutamate. The general solution of the equation is

This equation represents the method used for determining whether GABA flux is underestimated or overestimated by the misapplication of eqn. (1) to a system in which a glutamate precursor is radioactively labelled and glutamate is metabolically compartmented. The method does not however allow an evaluation of the magnitude of

$$a'_1 = a'_1(0) \exp(-C'_1 t) + a_6 - \dot{a}_6/C'_1 + \ddot{a}_6/(C'_1)^2 - \ddot{a}_6/(C'_1)^3 + \dots - [a_6(0) - \dot{a}_6(0)/C'_1 + \ddot{a}_6(0)/(C'_1)^2 - \ddot{a}_6(0)/(C'_1)^3 + \dots] \exp(-C'_1 t) \quad (4)$$

error in any particular case and the system of Scheme 1(b) was therefore implemented on an analogue computer to allow a general investigation of error magnitudes. In this system the amount of glutamate constituting each subpool is determined by the ratio of its formation flux to its utilization rate constant; the general case was characterized on the computer by two parameters, n and N , where n is the ratio of the formation fluxes into the two pools and N is the ratio of their utilization rate constants. The ratio of the two glutamate pool sizes is N/n and the time-course of radioactivity in the two pools was observed for a number of combinations of these parameters and for a variety of input functions representing specific radioactivity of the glutamate precursor. Under any particular conditions the error invoked by misapplying eqn. (1) to this system is given by

$$K_u/K_c = \dot{R}_2/[K_c(R_1/S_1 - R_2/S_2)] \quad (6)$$

To allow observation of this error the analogue computer programme was extended to give the functions $\dot{R}_2$ and $K_c(R_1/S_1 - R_2/S_2)$ during computation.

Method of evaluation of GABA flux from GABA perturbation values. Since after the uptake of GABA by brain slices there is little release of GABA and since there is no recirculation of ^{14}C into the GABA pool, the equation the attrition of [^{14}C]GABA is seen from Scheme 1(a) to be

$$\dot{R}_2 = -C_2 R_2 \quad (7)$$

With an analogue computer, solutions for R_2 from eqn. (7) were compared as functions of time with the concurrent output from a variable-diode function generator that had been set to represent the [^{14}C]GABA experimental results. The difference between the R_2 and experimental functions was used, after being squared and integrated, to provide an error function that could be minimized by incrementing the value of C_2 on the computer. The value of C_2 corresponding to minimum error function was adopted as the correct value. This type of procedure was also applied in matching other experimental

Since the respiration rate of the brain slices is a known constant the total flux of the tricarboxylic acid cycle may be allocated a constant value ϕ . Hence the unlabelled flux of oxaloacetate formation (through the reaction catalysed by α -oxoglutarate dehydrogenase) may be represented by the constant K_6^* where

$$K = C_6^* S_6 = \phi - K_1$$

The equations for the radioactivities and quantities of oxaloacetate and aspartate may thus be written

$$\dot{R}_4 = C_2 R_2 - C_4 R_4 + C_5 R_5 - C_4^* R_4 \quad (9)$$

$$\dot{R}_5 = C_4 R_4 - C_5 R_5 \quad (10)$$

$$\dot{S}_4 = \phi - K_1 + C_2 S_2 - C_4 S_4 + C_5 S_5 - C_4^* S_4 \quad (11)$$

$$\dot{S}_5 = C_4 S_4 - C_5 S_5 \quad (12)$$

In eqns. (9) and (11), the functions $C_2 R_2$ and $C_2 S_2$ are obtainable on the analogue computer from the established solutions of eqns. (7) and (8). Since the constants ϕ and K_1 are also known, any particular set of solutions of eqns. (9)–(12) is dependent upon the remaining unknown parameters C_4 , C_5 and C_4^* . A method for the evaluation of these parameters is by adjustment of their values to produce computer solutions for R_5 and S_5 that are in best agreement with the experimental results for the time-courses of radioactivity and quantity of aspartate respectively.

Method of assessing errors due to GABA compartmentation. The method described above for evaluation of GABA flux is based on the assumption that GABA is not metabolically compartmented but recent results have suggested that the assumption may not in fact be valid (Machiyama *et al.* 1970). Accordingly, equations have been derived that represent a general case of GABA compartmentation and these provide a method for assessing the error in evaluation of GABA flux that may arise with the assumption of homogeneity. Analytical solutions for the radioactivities in the two GABA pools of the system shown in Scheme 1(c) may be written:

$$R_2'(g-f) = [R_2'(0)(C'' + C_2'' - f) + R_2''(0)C'']e^{-ft} - [R_2'(0)(C' + C_2' - g) + R_2''(0)C']e^{-gt} \quad (13)$$

$$R_2''(g-f) = [R_2''(0)(C' + C_2' - f) + R_2'(0)C']e^{-ft} - [R_2''(0)(C' + C_2' - g) + R_2'(0)C']e^{-gt} \quad (14)$$

results and, in general, the resolution of parameter values that could be obtained was found to be less than 1% and the overall computer accuracy was in all cases within 0.1%.

The equation determining the quantity of GABA at any time, on the assumption of an uncompartmented system, is

$$\dot{S}_2 = C_1 S_1 - C_2 S_2 \quad (8)$$

In this equation, C_2 is predetermined by matching eqn. (7) to results for [^{14}C]GABA as already described and, since the quantity of glutamate remained constant for 90 min after GABA uptake, the flux $C_1 S_1$ was taken as a constant, K_1 , over the same period. The value of K_1 was found, by using the type of computer method already described, by matching the solution of eqn. (8) to the experimentally determined time-course of the amount of GABA.

$$\text{where} \quad f + g = C_2' + C_2'' + C' + C'' \quad (15)$$

$$\text{and} \quad fg = C_2' C_2'' + C_2' C' + C_2'' C' \quad (16)$$

If now the [^{14}C]GABA results are matched, by using the analogue computer, to a function of the form:

$$R_2 = Fe^{-ft} + Ge^{-gt}$$

then conjugate values of the constants F , f , G and g may be determined.

Since R_2 is the sum of R_2' and R_2''

$$F(g-f) = [R_2'(0)(g - C_2') + R_2''(0)(g - C_2'')] \quad (17)$$

$$\text{and} \quad G(f-g) = [R_2'(0)(f - C_2') + R_2''(0)(f - C_2'')] \quad (18)$$

Analytical solutions for the quantities of substrate in the two GABA pools of the system shown in Scheme 1(c) may be written

$$S'_2 = S'_2(\infty) + \{[S'_2(0) - S'_2(\infty)](C'' + C'_2 - f) + [S''_2(0) - S''_2(\infty)]C''\}e^{-f t} / (g - f) - \{[S'_2(0) - S'_2(\infty)](C'' + C'_2 - g) + [S''_2(0) - S''_2(\infty)]C''\}e^{-g t} / (g - f) \quad (19)$$

$$S''_2 = S''_2(\infty) + \{[S'_2(0) - S'_2(\infty)](C' + C'_2 - f) + [S'_2(0) - S'_2(\infty)]C'\}e^{-f t} / (g - f) - \{[S'_2(0) - S'_2(\infty)](C' + C'_2 - g) + [S'_2(0) - S'_2(\infty)]C'\}e^{-g t} / (g - f) \quad (20)$$

Eqs. (15)–(20) comprise a set of five independent equations that, however, involve the variables S'_2 and S''_2 together with nine unknown constants. The further equations necessary to allow the evaluation of these constants may be obtained by making the following assumptions.

(1) The experimental evidence suggesting the presence of GABA compartmentation arises in the specific and rapid release by K^+ of about one-third of the total GABA from incubated slices of brain cortex. This observation may be the result of total release from one GABA pool augmented by a 4% release from another (Machiyama, Balázs & Richter, 1967; Machiyama *et al.* 1970). The experiments in which GABA release was observed differ only from those reported in the main paper (Balázs *et al.* 1970) by the addition of K^+ at an elapsed time of 20 min. Comparison of the time-courses of GABA and $[1-^{14}C]$ GABA contents in the absence and presence of K^+ then allows estimates of the radioactivities and contents of the two GABA pools at 20 min to be made. The values relating to a 200 mg slice are: $R'_2(20) = 15000$ c.p.m., $R''_2(20) = 50000$ c.p.m., $S'_2(20) = 0.13 \mu\text{mol}$ and $S''_2(20) = 0.51 \mu\text{mol}$. The substitution of values for a time of 20 min in eqns. (13) and (14) gives only one further independent equation and the substitution of the corresponding values in eqns. (19) and (20) serves to remove the unknown variables S'_2 and S''_2 .

(2) In the experiments described in the preceding paper (Balázs *et al.* 1970) the quantity of GABA increased *post mortem* owing to the cessation of utilization caused by anoxia. If it is assumed that the transfer of GABA between its pools is inhibited during this period, the post-mortem increase of GABA in each pool may be taken as proportional to its formation flux. From Scheme 1(c) these formation fluxes may be derived as

$$K'_1 = S'_2(\infty)(C' + C'_2) - S''_2(\infty)C''$$

$$\text{and } K''_1 = S''_2(\infty)(C'' + C'_2) - S'_2(\infty)C'$$

In addition to the post-mortem increase, the further rise in GABA content of the two pools, owing to uptake from the medium, may be expressed in terms of the radioactivity values reached in each pool and the specific radioactivity of the GABA in the medium. The total increase of GABA in the two pools may thus be written

$$S'_2(0) - S'_2(\infty) = P[S'_2(\infty)(C' + C'_2) - S''_2(\infty)C''] + R'_2(0)/(R/S) \quad (21)$$

$$S''_2(0) - S''_2(\infty) = P[S''_2(\infty)(C'' + C'_2) - S'_2(\infty)C'] + R''_2(0)/(R/S) \quad (22)$$

where P is a constant and (R/S) represents the specific radioactivity of the medium.

(3) Finally, if the steady-state value for the total GABA contained in the 200 mg slice is obtained by extrapolation of experimental results, then

$$S'_2(\infty) + S''_2(\infty) = 0.33 \mu\text{mol} \quad (23)$$

Eqs. (15)–(23) now constitute a set of eight independent equations involving nine unknown constants and the latter may be evaluated upon the specification of one

further system equation. The total GABA flux under such conditions may then be deduced from the evaluated constants and compared with values obtained by other means.

RESULTS

Errors in evaluation of GABA flux due to glutamate compartmentation. If the specific radioactivity of the precursor of glutamate (a_6) is assumed to be a step function, then eqn. (5) simplifies to

$$K_c \leq K_u \text{ when } (C'_1 - C''_1)[a_6(0) - a_1(0)] \times [\exp(-C'_1 t) - \exp(-C''_1 t)] \leq 0$$

Therefore $K_c \leq K_u$ when $a_6(0) \geq a_1(0)$. Thus for all sudden increases in the radioactivity of the precursor of glutamate, the GABA flux (K_u) is an overestimate of the true flux (K_c). For sudden decreases in radioactivity of glutamate formation the converse is true.

If the specific radioactivity of the precursor of glutamate is assumed to increase linearly from zero time, then eqn. (5) simplifies to

$$K_c \leq K_u \text{ when } (C'_1 - C''_1)a_6\{[1 - \exp(-C'_1 t)]/C'_1 - [1 - \exp(-C''_1 t)]/C''_1\} \leq 0$$

Therefore $K_c \leq K_u$ when $a_6 \geq 0$. Thus for all linear increases in the radioactivity of the precursor of glutamate, the GABA flux (K_u) is an overestimate of the true flux (K_c). For linear decreases in the radioactivity of the precursor of glutamate the converse is true.

In the more general case, where the radioactivity of the glutamate precursor is not described by simple functions of time, eqn. (5) is still valid but is of a form that does not allow generalized conclusions to be reached. However, implementation of the system shown in Scheme 1(b) on an analogue computer allowed investigation of general time-functions representing specific radioactivity of the

glutamate precursor; the functions consisted of rising and subsequently falling phases. These investigations showed that in the initial stages when the radioactivities of both glutamate pools were rising, the GABA flux evaluated on the assumption of an uncompartmented system was an overestimate.

Fig. 1. Analogue-computer plots illustrating the error in evaluation of GABA flux arising from the misapplication of eqn. (1) to the system shown in Scheme 1(b). The ratio of glutamate-formation fluxes (n) is unity and the ratios of glutamate-utilization rate constants (N) are 1, 2 and 5 respectively. The quantities of GABA and glutamate are nominally taken as 0.5 and 1.5 μmol respectively and C_2 is taken as 0.02/min. —, functions R_2 ; ----, functions $K_c(R_1/S_1 - R_2/S_2)$; both are plotted to the same arbitrary scale. The error in evaluation of GABA flux at any times is shown by eqn. (6) to be the ratio of the ordinates of the two conjugate curves at that time.

When the radioactivity of both pools was subsequently decreasing an underestimate was obtained. When the radioactivity of one pool was rising while that of the other was falling the evaluation of GABA flux passed from overestimate to underestimate.

The analogue-computer simulation of the system shown in Scheme 1(b) was also adapted to evaluate the K_u/K_c ratio as described by eqn. (6). This ratio gives, in any specified case, the actual magnitude of error involved in misapplying eqn. (1) to a system in which glutamate precursors are labelled and in which glutamate is a metabolically compartmented substrate. A typical set of results is given in Fig. 1, which shows the response to a step increase in the specific radioactivity of the precursor of glutamate. Under the conditions stated for this figure, when N has a value of 2 the GABA flux is overestimated by 17% at 90 min, and when N has a value of 5 the GABA flux is overestimated by 150% at 90 min. Even larger overestimates of GABA flux are obtained in simulated systems where the fluxes of glutamate formation are not assumed to be equal.

Evaluation of GABA flux from GABA perturbation results. Computer simulation, as described in the Methods section, allowed the experimental results for the attrition of $[1-^{14}\text{C}]\text{GABA}$ and the total quantity of GABA to be matched to eqns. (7) and (8) respectively. The value of C_2 was found to be 0.019/min (see Fig. 5 of Balázs *et al.* 1970) and the value for K_1 was 0.0315 $\mu\text{mol}/\text{min}$ per g wet wt. (see Fig. 2 of Balázs *et al.* 1970).

The respiration rate of the brain tissue slices is given in the preceding paper (Balázs *et al.* 1970) as

$72.8 \pm 0.25 \mu\text{mol}$ of oxygen/h per g wet wt., from which the value of ϕ in eqn. (11) is 0.401 $\mu\text{mol}/\text{min}$ per g wet wt. In attempting to match the computer solutions of eqns. (10) and (12) to the experimental results for the radioactivity and total quantity of aspartate respectively it was found that a wide range of conjugate sets of values for the remaining unknown constants (C_4 , C_5 and C_4^*) agreed equally well with the results. A value of 4 $\mu\text{mol}/\text{g}$ wet wt. for the steady-state content of oxaloacetate was therefore taken from the literature (Goldberg, Passonneau & Lowry, 1966) which, since $\phi = 0.40 \mu\text{mol}/\text{min}$ per g wet wt. implies a value for C_4^* of 100/min. With this value the best simultaneous computer solutions of eqns. (10) and (12) were determined by adjusting the rate constants C_4 and C_5 (See Fig. 6 of Balázs *et al.* 1970). Similar solutions were obtained by using the foregoing method when the value of oxaloacetate content was varied between 0.5–8 nmol/g; the system thus seems relatively insensitive to the assumed value of oxaloacetate content.

Effect of GABA compartmentation on evaluation of GABA flux. In the latter part of the Methods section, equations have been described that allow assessment of the effect of possible GABA compartmentation on the evaluation of GABA flux as described above. The method requires that the attrition of $[1-^{14}\text{C}]\text{GABA}$ can be described as the sum of two decaying exponentials and the present results are based upon the selection of the particular function

$$R_2 = 40e^{-0.037t} + 60e^{-0.014t}$$

These values were chosen since, as shown by using the analogue computer, they provide an agreement with [$1\text{-}^{14}\text{C}$]GABA results that is just acceptable, have comparable base values and yet have considerably different exponents. The above numerical function may thus be expected to embody an extreme interpretation of experimental results in terms of the significance of compartmentation. Use of this function gives corresponding values of F , f , G and g in the eight independent expressions of eqns. (15)–(23) but, since the equations involve nine unknown parameters, a unique set of values cannot be obtained. However, when $R'_2(0)$ is treated as an independent parameter it is found that when $R'_2(0)$ exceeds 13430 c.p.m. the resultant set of parameter values always includes a negative value for one or other of the reaction rate constants. The values of GABA flux given by the remaining numerical solutions were within the limits of 0.0300 and 0.0360 $\mu\text{mol}/\text{min}$ per g wet wt.

An independent evaluation of GABA flux, assuming that the experimental results on [$1\text{-}^{14}\text{C}$]GABA and total GABA quantity arose from a compartmented system, was made by using an analogue-computer simulation based on the system shown in Scheme 1(c). By this means it was estimated that the total GABA flux was within the range of $0.0317 \pm 0.004 \mu\text{mol}/\text{min}$ per g wet wt.

DISCUSSION

The present results provide an estimate for the GABA flux in guinea-pig cerebral cortex *in vitro* of 0.0315 $\mu\text{mol}/\text{min}$ per g wet wt. or 8% of the total tricarboxylic acid cycle flux. This figure is at variance with the larger value of 40% obtained by McKhann *et al.* (1960), which is frequently quoted in the literature. However, the method by which this larger value was obtained used eqn. (1) in misapplication to a system in which glutamate is now known to be metabolically compartmented. The results of computer simulation, illustrated by Fig. 1, now show that gross errors in estimation of GABA flux may arise in the interpretation of experiments with labelled precursors of glutamate, if glutamate compartmentation is not taken into account. Further, the analytical mathematical

approach shows that under such conditions calculations based on the initial rising phase of GABA radioactivity will, under all conditions, provide an overestimation of GABA flux. On the basis of these mathematical approaches, the present lower value for GABA flux seems to be well supported.

The computer results given in Figs. 2 and 5 of the preceding paper (Balázs *et al.* 1970) show that the time-courses of both [$1\text{-}^{14}\text{C}$]GABA radioactivity and total quantity of GABA are well described on the assumption of a homogeneous GABA system. However, it has also been found by using the analogue computer that inherent experimental errors give a scatter to the plotted results that allows a wide variety of double-exponential functions to be equally well fitted to the experimental time-courses. Thus analysis of the results in the preceding paper (Balázs *et al.* 1970) does not of itself warrant the assumption that GABA is metabolically compartmented, but neither does it show inconsistency with the metabolic compartmentation of GABA postulated from other biochemical evidence. Whether or not GABA is assumed to be compartmented the present results show little variation in the implied value of GABA flux; computer simulation methods indicate the probable limits to be $\pm 15\%$ whereas the assumption of an extreme compartmented system (just acceptable when compared with experimental results) gives flux values that are within -5% to $+20\%$.

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