able with the values of the hypothermic rats of group II, the lactate concentration of the blood invariably increases to about 300 to 400% of the control values (HUCKABEE[6]). Since no increase occurs in the hypothermic rats at a pH of 7.60, the hydrogen ion concentration itself is apparently not correlated with the changes in lactate concentration in hypothermia. However, the lactate concentration increases also in hypothermia if the ventilation is enhanced and the OH^-/H^+ ratio is raised. It therefore seems conceivable that the increase in lactate caused by hyperventilation is correlated with the relative alkalinity rather than with the absolute value of the hydrogen ion concentration.

Zusammenfassung. Der durch Hyperventilation ausgelöste Milchsäureanstieg bleibt bei vergleichbarer Senkung des pH in Hypothermie aus, solange dabei der OH^-/H^+-Quotient bzw. die relative Alkalinität nach RAHN[2] konstant bleibt. Stärkere Hyperventilation, die zu einer Zunahme des OH^-/H^+-Quotienten führt, löst dagegen auch in Hypothermie einen Milchsäureanstieg aus.

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Binding of γ-Aminobutyric Acid and other Amino Acids by Particulate Fractions of Developing Rat Brain[1]

In previous reports it has been shown that γ-aminobutyric acid (GABA) 'binds' rapidly to subcellular particles of brain by a Na^+-dependent mechanism which does not require enzymatic action or cellular energy[2–7]. It has also been shown that Na^+-dependent GABA binding is maximal at about pH 7.3[4,8] which is approximately the isoelectric point (pI) for GABA[9]; i.e., GABA binding is maximal at physiological pH when it is present as a 'zwitterion' and hence this binding may require two oppositely-charged membrane sites. Some evidence has been provided that other amino acids may be bound by Na^+-dependent mechanisms to brain particles[4], but no studies have been carried out on these processes in developing brain. In the present study, the Na^+-dependency of binding of GABA has been compared with that of other amino acids in subcellular particles prepared from rat brain during development.

Male, Sprague-Dawley rats, 1–75 days old (Indianapolis Lab. Supply Co.) were purchased as littermates except for those which were 35–36 or 74–75 days old. After decapitation, cerebral hemispheres were excised rapidly, weighed and homogenized immediately in 10 volumes of ice-cold isosmotic sucrose solutions containing 0.1 or 0.2 μCi/ml of U[^{14}C] D-sucrose (New England Nuclear Corp.: 505 mCi/mmole) + 0.25–0.45 μCi/ml of the radioactive amino acids [2, 3-^{3}H]γ-aminobutyric acid, 2 Ci/mmole; [^{3}H] L-glutamic acid, 1.9 Ci/mmole; [2-^{3}H] glycine, 11.1 Ci/mmole,

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[2] K. A. C. ELLIOTT and N. M. VAN GELDER, J. Physiol., Lond. *153*, 423 (1960).

[3] K. SANO and E. ROBERTS, Biochem. biophys. Res. Commun. *4*, 358 (1961).

[4] K. A. C. ELLIOTT, R. T. KHAN, F. BILODEAU and R. A. LOVELL, Can. J. Biochem. *43*, 407 (1965).

[5] S. VARON, G. WEINSTEIN, T. KAKEFUDA and E. ROBERTS, Biochem. Pharmac. *14*, 1213 (1965).

[6] K. KURIYAMA, E. ROBERTS and J. VOS, Brain Res. *9*, 231 (1968).

[7] K. A. C. ELLIOTT, Br. med. Bull. *21*, 70 (1965).

[8] K. SANO and E. ROBERTS, Biochem. Pharmac. *12*, 489 (1963).

[9] R. M. C. DAWSON, D. C. ELLIOTT, W. H. ELLIOTT and K. M. JONES, *Data for Biochemical Research*, 2nd edn. (Oxford University Press, Oxford 1969).

Table I. Differences between percentage distributions of ^{3}H-amino acids and ^{14}C-sucrose in P_1 fractions of developing rat brain

Post-partum age of rat (days)	(% ^{3}H-amino acid) minus (^{14}C-sucrose) in P_1 fraction			
	GABA	Glutamate	Glycine	Arginine
	Na^+-free homogenizing fluid			
1	0.6 ± 0.18	0.5 ± 0.15	0.2 ± 0.11[b]	1.9 ± 0.34[c]
11	0.4 ± 0.16	0.7 ± 0.10[a]	0.3 ± 0.06	1.6 ± 0.10[c]
15	0.3 ± 0.10	0.6 ± 0.13[c]	0.3 ± 0.10	1.6 ± 0.13[c]
21–22	0.5 ± 0.11	0.3 ± 0.13	0.2 ± 0.08[c]	1.4 ± 0.16[c]
35–36	0.5 ± 0.16	0.1 ± 0.10[c]	0.3 ± 0.09	2.2 ± 0.27[c]
74–75	0.5 ± 0.08	0.04 ± 0.10[c]	0.2 ± 0.08[c]	2.7 ± 0.45[c]
	Homogenizing fluid containing 40 m*M* NaCl			
1	2.4 ± 0.29	1.2 ± 0.21[c]	1.7 ± 0.26[a]	2.7 ± 0.35
11	5.4 ± 1.02	1.9 ± 0.39[c]	2.9 ± 0.29[c]	4.7 ± 0.43
15	5.2 ± 0.40	2.7 ± 0.44[c]	2.9 ± 0.16[c]	5.6 ± 1.33
21–22	7.5 ± 1.08	2.9 ± 0.50[c]	2.9 ± 0.14[c]	3.9 ± 0.69[c]
35–36	8.1 ± 0.54	3.2 ± 0.13[c]	2.7 ± 0.47[c]	3.5 ± 0.61[c]
74–75	6.7 ± 0.35	2.7 ± 0.30[c]	2.4 ± 0.22[c]	3.2 ± 0.34[c]

Means ± standard deviations of individual differences between ^{3}H and ^{14}C found in each pellet fraction; 4 pellets from 4 rat brains in all cases. [a] $p < 0.02$; [b] $p < 0.01$ and [c] $p < 0.001$, for comparisons between values for other amino acids versus GABA.

Table II. Differences between percentage distributions of ^{3}H-amino acids and ^{14}C-sucrose in P_2 fractions of developing rat brain

Post-partum age (days)	(% ^{3}H-amino acid) minus (% ^{14}C-sucrose) in P_2 fraction			
	GABA	Glutamate	Glycine	Arginine
	Na^+-free homogenizing fluid			
1	1.3 ± 0.22	1.6 ± 0.11	1.8 ± 0.22[a]	10.8 ± 0.63[b]
11	2.2 ± 0.21	3.6 ± 0.21[b]	5.0 ± 0.28[b]	19.4 ± 3.19[b]
15	1.5 ± 0.13	4.2 ± 0.45[b]	4.2 ± 0.15[b]	19.3 ± 0.72[b]
21–22	1.6 ± 0.30	3.2 ± 0.14[b]	3.9 ± 0.18[b]	18.0 ± 0.50[b]
35–36	1.6 ± 0.21	2.8 ± 0.29[b]	3.0 ± 0.13[b]	15.7 ± 0.30[b]
74–75	1.0 ± 0.32	2.2 ± 0.28[b]	2.9 ± 0.22[b]	13.7 ± 0.41[b]
	Homogenizing fluid containing 40 mM NaCl			
1	8.7 ± 1.14	2.6 ± 0.27[b]	3.4 ± 0.25[b]	5.9 ± 0.20[b]
11	17.2 ± 0.82	3.9 ± 0.34[b]	6.4 ± 0.44[b]	12.0 ± 0.29[b]
15	16.8 ± 1.51	4.6 ± 0.80[b]	4.1 ± 0.51[b]	6.2 ± 0.31[b]
21–22	8.7 ± 0.25	2.3 ± 0.39[b]	2.8 ± 0.31[b]	6.2 ± 0.47[b]
35–36	4.0 ± 0.73	1.4 ± 0.23[b]	1.8 ± 0.19[b]	3.3 ± 0.06
74–75	3.5 ± 0.27	1.2 ± 0.15[b]	1.7 ± 0.20[b]	3.1 ± 0.53

Means ± standard deviations of individual differences between ^{3}H and ^{14}C found in each pellet fraction; 4 pellets from 4 rat brains in all cases. [a] $p < 0.02$ and [b] $p < 0.001$, for comparisons between values for other amino acids versus GABA.

[^{3}H] L-arginine, 24.2 Ci/mmole; New England Nuclear Corp.) ± 40 mM NaCl. The pH of resultant homogenates was 6.8–7.2. The amounts of tritiated amino acids used accounted for < 1% of the total amino acids released from the tissue during homogenization and therefore these served only as 'tracer' substances. Aliquots of homogenates were centrifuged at 1,000 ×g, 10 min, 0 °C to obtain P_1 (crude nuclear pellet) and S_1 (first supernatant) fractions; re-centrifugation of aliquots of S_1 fractions at 17,500 ×g, 55 min, 0 °C provided the P_2 pellet (containing nerve endings and mitochondria) and the S_2 fraction (containing ribosomes, microsomes and cellular sap)[10]. All other procedures were carried out at 0–4 °C to prevent the catabolism of amino acids and their incorporation into protein, and all fractions were weighed on a microbalance. Radioactivity due to [^{14}C] sucrose (which provided an estimate of entrapped homogenizing fluid) and that due to [^{3}H] amino acids (which provided an estimate of their binding) was determined in 100–500 µl aliquots of soluble fractions and re-suspended pellets. Radioactivity in all fractions was monitored by a double-labelling procedure using a Packard Model 3375 scintillation counter and conversions of cpm to dpm and corrections for 'spillover' were made using quench and correlation curves (channels-ratio methods). A counting 'cocktail', described previously[11], permitted measurement of radioactivity with minimal quenching. Recoveries ranged from 92–100% for [^{14}C] and from 91–127% for [^{3}H] for centrifugations at 1,000 ×g, and from 91–103% for [^{14}C] and from 85–100% for [^{3}H] for centrifugations at 17,500 ×g. In all aliquots counted, > 1,000 dpm of [^{14}C] and [^{3}H] were present.

Data presented in the Tables are expressed as differences between the % [^{3}H] (due to amino acid) and % [^{14}C] (due to entrapped sucrose) where,

[10] E. G. GRAY and V. P. WHITTAKER, J. Anat., Lond. *96*, 79 (1962).
[11] F. V. DE FEUDIS, Res. Commun. chem. path. Pharmac. *2*, 189 (1971).

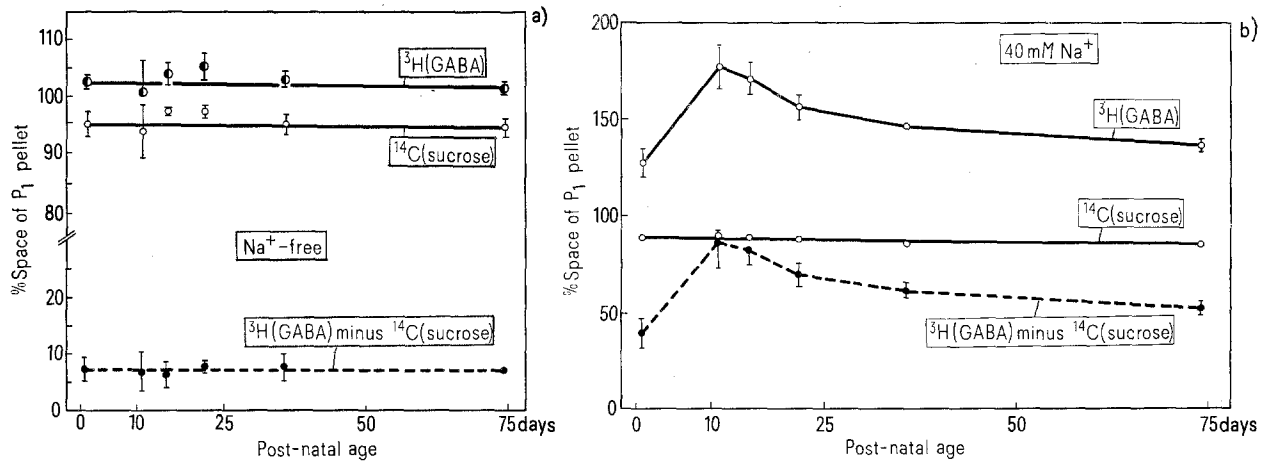

Fig. 1. Effects of Na^+ on '% spaces' of P_1 pellets prepared from developing rat brain (a) homogenization in the absence of added Na^+; (b) homogenization in medium containing 40 mM NaCl.

$$\% \text{ dpm in pellet} = \left(\frac{\text{dpm in pellet}}{\text{dpm in pellet} + \text{supernatant fluid}}\right)(100).$$

To calculate '% spaces' (for data shown in the figures), the following equation was used:

$$\% \text{ space of pellet} = \left(\frac{\text{dpm/g of pellet}}{\text{dpm/g of supernatant}}\right)(100).$$

% Space measurements provide theoretical values for the % of the total pellet weight which contains radioactivity at the same concentration as that in the corresponding supernatant fraction. This method thus provided an additional estimate of the extent to which [^{3}H] GABA was bound to tissue particles during various stages of brain development. Addition of 40 mM NaCl to homogenizing fluids caused increases in P_1 pellets and corresponding decreases in P_2 pellets, but these changes were corrected for by monitoring the extent to which [^{14}C] sucrose was occluded in pellets.

Striking changes in the binding of all the amino acids were evident during development. In Table I it is shown that negligible amounts of GABA, glycine and glutamate were bound to P_1 fractions in the absence of Na^+, but that a considerable amount of arginine was bound. In the presence of added Na^+ both glycine and glutamate were bound to a lesser extent than GABA over the whole age range studied but in brain particles from younger rats arginine was bound similarly to GABA. It is noteworthy that addition of Na^+ caused increased binding of all amino acids studied to P_1 fractions. Data in Table II indicate that in the absence of added Na^+, glutamate, glycine and arginine were all bound to a greater extent than GABA to P_2 fractions of rats of all ages. But, with added Na^+, GABA was bound more than the other amino acids. Binding of arginine to the P_2 fraction occurred to the greatest extent in the absence of Na^+ and the binding of glycine and glutamate were hardly affected by addition of Na^+. Perhaps the most interesting finding (Table II) was that the binding of all amino acids studied was maximal in P_2 fractions (which contain nerve endings) prepared from the brains of 1-, 11- and 15-day-old rats, both in Na^+-free media and in the presence of added 40 mM Na^+.

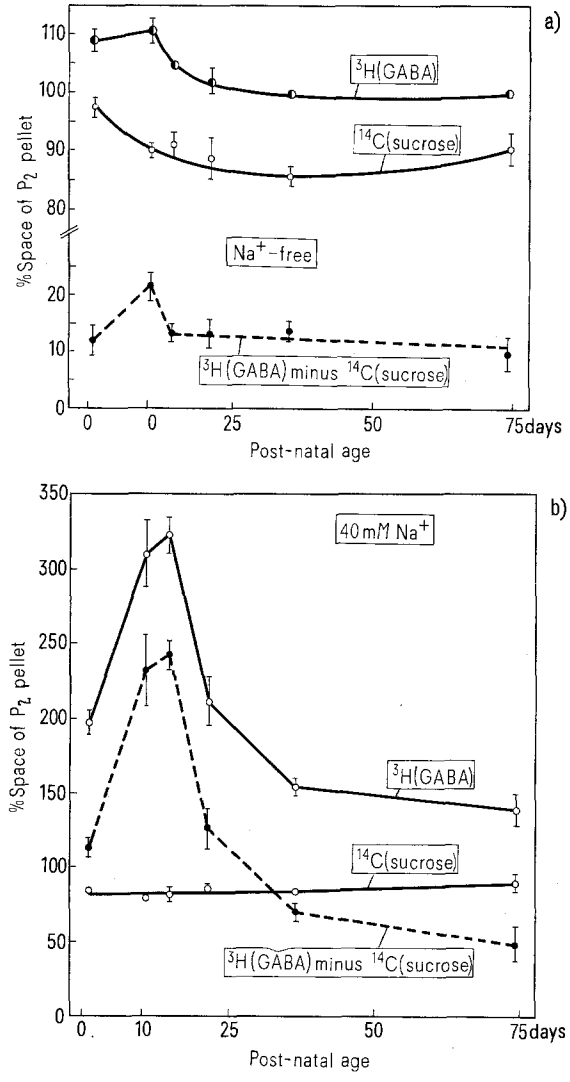

Fig. 2. Effects of Na^+ on '% spaces' of P_2 pellets prepared from the rats of different ages. (a) homogenization in the absence of added Na^+; (b) homogenization in the presence of 40 mM NaCl.

In Figure 1a it is shown that a slight amount of GABA is bound to P_1 fractions in the absence of added Na^+ but that this binding is not age-dependent. In the presence of 40 mM Na^+, % sucrose spaces remained similar to those shown in Figure 1a, but appreciably more GABA was bound and binding was maximal in the 11–15-day-old rats (Figure 1b). Results shown in Figure 2a indicate that slightly more GABA is bound to the P_2 than to the P_1 pellet in the absence of Na^+. A striking increase in the capacity for GABA binding to P_2 fractions of brains of 11- and 15-day-old rats occurred in the presence of 40 mM Na^+, while % sucrose spaces remained relatively constant over the whole age range.

The results presented herein provide evidence that GABA binding is more dependent on Na^+ than the binding of arginine, glycine or glutamate. Though arginine binding appeared to be increased in the P_1 fraction by addition of Na^+, it was actually decreased by added Na^+ in the P_2 fraction. The binding of glycine and glutamate to the P_2 fractions of developing rat brain were hardly changed by addition of Na^+. All amino acids studied were bound maximally to P_2 fractions prepared from 11- and 15-day-old rats and binding decreased with further maturation. It is interesting that maximal binding of amino acids to P_2 fractions occurs during a period of growth in which synaptic development[12] and brain excitability[13] are also maximal.

Résumé. La rétention de l'acide γ-aminobutyrique dans des particules subcellulaires du cerveau dépend davantage du Na^+ que ce n'est le cas des autres acides aminés. La rétention des acides aminés dans le cerveau est en fonction de l'âge des rats.

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[12] G. K. Aghajanian and F. E. Bloom, Brain Res. *6*, 716 (1967).

[13] P. S. Timiras, A. Vernadakis and N. M. Sherwood, in *Biology of Gestation*, Ed. (N. S. Assali, Academic Press, New York 1968), p. 261.

[14] Thanks are due to Mr. Paul Madtes, Mr. John Marks and Mrs. Janice Leonard for technical assistance, to Mrs. Jeanne Wilson for typing this manuscript and to Mr. Phil Wilson for the art work.