Distribution of H^3-Glycine and H^3-L-Glutamate in Synaptosomal Subpopulations after in vitro Uptake into Cat Dorsal and Ventral Spinal Cord Slices

In mammalian spinal cord, glycine and glutamate have been considered as potential inhibitory and excitatory neurotransmitters, respectively[1,2]. Apart from the electrophysiological evidence, specific high affinity transport mechanisms have been found for both amino acids, with Km-values of the order of 10^{-5} M[3-6]. The distinct regional distribution of endogenous glycine and glutamate in grey and white matter, and in dorsal and ventral regions, has also suggested a specific function for these amino acids in the spinal cord[7-9]. After fractionation on a linear sucrose gradient of spinal cord slices incubated with labelled glycine and glutamate, BALCAR and JOHNSTON[6] found an accumulation of both amino acids in the region of the synaptosomes (around 1.0 M sucrose). IVERSEN and BLOOM[10] have suggested that in spinal cord different transmitters are accumulated by separate populations of synaptosomes. We describe here the fractionation of synaptosomes from the dorsal and ventral halves of cat spinal cord on a discontinuous sucrose gradient, after uptake of H^3-glycine and H^3-L-glutamate in vitro, distinctive distribution patterns for these two potential transmitters being obtained. A preliminary report has appeared in this journal[11].

Table I. Tissue/medium ratios for H^3-glycine and H^3-L-glutamate in dorsal and ventral halves of cat spinal cord after 10 min uptake into tissue slices at 25 °C

	Dorsal	Ventral	Dorsal/ventral ratio
H^3-L-glutamate	0.489	0.351	1.39
H^3-glycine	1.753	1.113	1.57
Glycine/glutamate ratio	3.58	3.17	

Calculated as cpm per g fresh tissue/cpm per ml incubation medium. Averages of 4 experiments each.

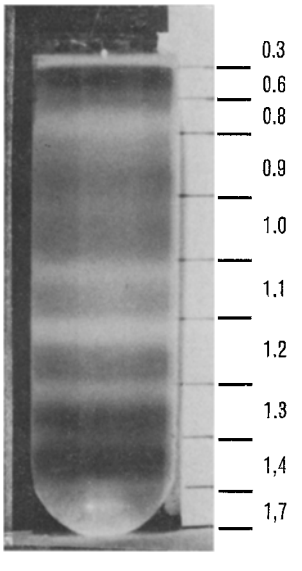

Fig. 1. Photograph of 10-step sucrose density gradient after completion of 90 min/60,000 g centrifugation. 0.5 ml nuclei-free homogenate of cat spinal cord was applied (see Methods). Bands between 0.6–0.9 M sucrose contain myelin and membrane fragments. 4 bands between 1.0–1.3 M sucrose contain mostly synaptosomes. 2 bands in 1.4 and 1.7 M sucrose contain mostly mitochondria.

Materials and methods. Adult cats weighing 1.5–2.1 kg were anaesthetized with 40 mg Nembutal/kg i.p. and killed by aortic transsection. The spinal cord was taken from lumbar segments 6 and 7, and carefully dissected into dorsal and ventral halves. 120 mg slices (1.0 × 0.5 mm) were incubated for 10 min at 25 °C in 2.0 ml Krebs-Henseleit buffer, pH 7.4, with 5×10^{-7} M H^3-glycine or H^3-L-glutamate (Radiochemical Centre, Amersham). After washing with 5 ml 0.3 M sucrose, the slices were homogenized (1:10, w/v) in this medium, the nuclei were sedimented at 184 g/10 min, and 0.5 ml of the supernatant was layered on top of a 10-step discontinuous sucrose density gradient ranging from 0.6 M to 1.7 M sucrose (for details see[12]). After 90 min centrifugation at 60,000 g, the gradient was collected in 30 fractions of 0.42 ml each. 0.1 ml of each fraction was mixed with 0.1 ml hyamine hydroxide and added to 15 ml scintillator mixture for liquid scintillation counting. The distribution of the material on the gradient was monitored during collection by UV-absorption at 254 nm, and samples from each major band were collected for electron microscopic identification. The photograph in Figure 1 shows the distribution of the material on the gradient after centrifugation. Protein was determined by the method of LOWRY et al.[13].

Results and discussion. The tissue/medium ratios for the whole slices (Table I) show that in both dorsal and ventral spinal cord the initial uptake rate for glycine is 3 times higher than that of glutamate. For both amino acids, uptake was higher in the dorsal region. This may be partly due to the slightly higher protein content per g tissue weight, which was 49.7 mg/g in the ventral half and 56.3 mg/g in the dorsal half (average of 8 experiments), giving a ratio of 1.13. In a separate experiment, the tissue/medium ratios for glycine were determined in grey and white matter: ventral grey 3.3, dorsal grey 4.3, ventral white 0.8, dorsal white 1.1. The dorsal/ventral ratios are similar to those for the combined slices. As was to be expected, there is a 3–4-fold higher uptake rate in grey matter than in white.

The distribution of H^3-glycine and H^3-L-glutamate on the 10-step sucrose gradient is shown in Figure 2. The 4 main bands in the region of the synaptosomes (fractions 8–23, 1.0–1.3 M sucrose) correspond to those visible in these positions in Figure 1. The proportion of H^3-glycine

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to H^3-L-glutamate varies from band to band, being greater in the heavier synaptosomes. Our stepgradient thus shows that the synaptosomal populations actively transporting glycine and L-glutamate are not identical. This supports the autoradiographic and uptake data of IVERSEN and BLOOM[10]. By fractionating the entire (nuclei-free) homogenate instead of a resuspended washed 100,000 *g* pellet[6], we obtained a more detailed picture of the relative quantitative distribution of glycine and glutamate uptake. The quantitative analysis of our data in Table II shows that a very high percentage of the amino acids is taken up by cellular components other than synaptosomes, which after homogenization appear at the top of the gradient (less than 0.9 *M* sucrose), fractions 24–30. This suggests that, inspite of the low tissue/medium ratios in white matter in the spinal cord, the large proportion of glial cells in this tissue plays an important part, besides the neurones, in the removal of glycine and glutamate from extracellular space. The dorsal/ventral uptake ratios are close to unity for this cell compartment, suggesting that only unspecific transport mechanisms are involved.

In two experiments, H^3-Gaba was included. The tissue/medium ratios were 1.47 and 1.77 (ventral), and 3.04 and 2.58 (dorsal), giving dorsal/ventral uptake ratios of 2.07 and 1.45 similar to our values for H^3-glycine and H^3-glutamate (Table I). The fractionation pattern for Gaba was similar to that for glutamate in Figure 2.

While the dorsal/ventral ratios for glutamate uptake in the slices (Table I) and in the synaptosomal fractions (Table II) are roughly similar to that of the endogenous free glutamate pool, they are reversed for glycine[7–9]. Both dorsal and ventral regions of cat spinal cord have high glycine levels compared to cerebral cortex, but the ventral level is higher by a factor of about 1.2. Our findings seem to indicate that the uptake rates for 'high specificity' kinetics (which can be expected to dominate for amino acids at 5×10^7 *M*) are not necessarily governed by the level of the endogenous free pool of the substance being transported. A much more detailed regional analysis of endogenous concentrations and of uptake kinetics would be required to decide this important question.

Fractionation of subcellular particles from spinal cord has been carried out on a linear sucrose gradient[6], and on 2-step discontinuous gradients[14,15]. The synaptosomes

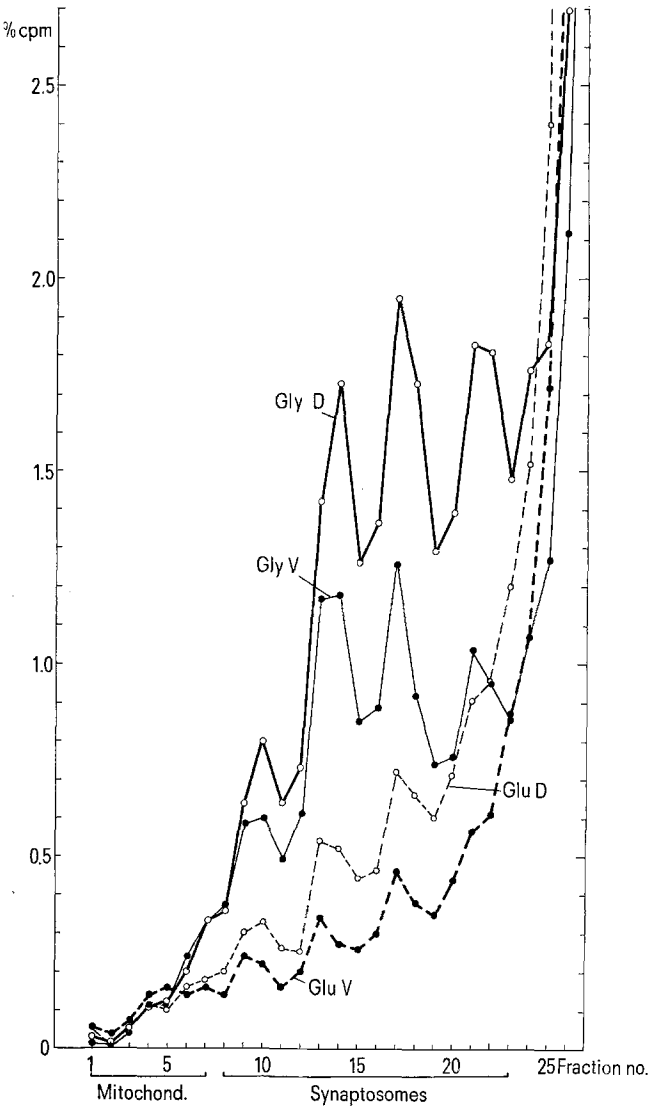

Fig. 2. Distribution of H^3-glycine and H^3-L-glutamate on a 10-step discontinuous sucrose density gradient after 10 min uptake at 25 °C into slices of dorsal and ventral cat spinal cord. The percentage of the total label recovered from the gradient, appearing in each fraction is given. Each point is the average of 4 independent determinations. Gly D, glycine, dorsal; Gly V, glycine, ventral; Glu D, glutamate, dorsal; Glu V, glutamate, ventral.

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Table II. Percent distribution of H^3-glycine and H^3-L-glutamate on a discontinuous sucrose density gradient after 10 min uptake into slices of dorsal and ventral cat spinal cord at 25 °C

Fractions		1–7 (mitochondria)	8–23 (synaptosomes)	24–30
H^3-glycine % cpm	(dorsal)	0.9 ± 0.2	20.4 ± 5.1	78.6 ± 5.3
	(ventral)	0.9 ± 0.2	13.4 ± 2.6	85.7 ± 2.7
H^3-L-glutamate % cpm	(dorsal)	0.6 ± 0.2	9.1 ± 0.6	90.3 ± 0.6
	(ventral)	0.8 ± 0.2	5.9 ± 0.5	93.3 ± 0.7
Dorsal/ventral ratio	Glycine	1.00	1.52	0.91
	Glutamate	0.75	1.54	0.96
Glycine/glutamate ratio	(dorsal)	1.5	2.24	0.87
	(ventral)	1.1	2.27	0.91

The 30 fractions were grouped in 3 regions containing predominantly mitochondria, synaptosomes (in 4 bands), and membrane fragments, myelin and supernatant. Averages of 4 separate experiments for each amino acid are given.

were, however, obtained in a single band or peak without further differentiation. Our gradient, which subdivides the density region between 0.9 *M* and 1.4 *M* sucrose into 5 separate steps, permits a more detailed analysis of synaptosomal subpopulations (see also [12]). It appears that with such a gradient distinct subpopulations of synaptosomes can be partially separated.

Zusammenfassung. Für die Verteilung von H^3-Glycin und H^3-L-Glutamat auf einem 10-Stufen-Gradienten nach Aufnahme in Katzenrückenmarkschnittchen werden unterschiedliche Muster erhalten, die auf verschiedene Synaptosomenpopulationen schliessen lassen.

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DNA and RNA Concentration in the Liver of Japanese Quail (*Coturnix coturnix japonica*) During Growth

On the basis of results obtained in various studies, it may be concluded that DNA concentration in the liver and other organs and tissues is relatively stable. RNA concentration, on the other hand, sensitively reflects the changes in the physiological, but especially in the metabolic processes which are developed as a result of the action of various internal and external factors[1–5]. During animal growth, as well as at its end, great changes in protein metabolism occur. DNA concentration in the liver of growing animals does not change[2], whereas the content of RNA is closely correlated with the intensity of protein synthesis[1–3]. However, on the basis of some experiments it has been found that in growing animals there is a significant change in DNA concentration in the liver[6]. Our experimental work, which deals with the progress as well as with the changes of DNA and RNA concentration in the liver of Japanese quail from the time of their hatching until they have reached sexual and body maturity, is intended as a contribution to this specific problem.

Materials and methods. The problem was investigated in Japanese female quail. These were kept in very good condition and fed a mixture of protein containing 28% of N-substances. Beginning with the day of their hatching until they had reached the age of 70 days, the quail were slaughtered in groups of 5 at intervals of 7 days and their liver DNA and RNA concentration was determined by Schneider's method[7]. The quail began laying eggs when they had reached the mean age of 48 days.

The values obtained from DNA and RNA concentration have been evaluated by a mathematical-statistical method in the following way: The homogenity of variances among the age groups was determined by Cochran's[8] G-test. If the G-test was not significant, the results were then evaluated by the analysis of a variance. If the G-test was significant, the inter-group differences were tested by Student's *t*-test[9], or in the case of heterogenity of the group variances by Welch's *t*-test[10].

Results and discussion. The values obtained from DNA concentration in the liver as well as from the live weight and the weight of the liver are shown in Table I. From the values it can be seen that DNA concentration does not change in the liver of the growing animals and is marked by a low variability. On the other hand, RNA concentra-

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Table I. Live weight, weight of liver and DNA concentration in the liver ($n = 5$)

Age (days)	Mean weight (g)		DNA concentration (μg/100 mg of tissue)	
	Quail	Liver	Mean ± S.E.	C.V. (%)
0	6.90	0.21	24.01 ± 0.09	0.77
7	16.23	0.57	24.17 ± 0.08	0.64
14	24.85	0.93	24.23 ± 0.11	0.91
21	46.57	1.64	24.27 ± 0.09	0.74
28	56.93	1.70	24.15 ± 0.12	0.96
35	84.87	2.85	24.26 ± 0.05	0.45
42	96.25	3.04	24.28 ± 0.05	0.40
49	117.92	3.47	24.26 ± 0.16	0.33
56	123.59	3.50	24.33 ± 0.03	0.28
63	117.42	3.37	24.25 ± 0.12	0.28
70	112.61	3.90	24.28 ± 0.08	0.67
G-test			not significant	
F-test			not significant	

n = number of animals in a group, *c.v.* (%) = coefficient of variation.