Taurine Uptake into Chick B Cells (43354)

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Abstract. **The objective of the present study was to determine whether chick B cells possess a specific transport system for taurine. The Bursa of Fabricius was isolated from newly hatched to 6-week-old chicks and an enriched fraction (86.2%) of B cells was isolated. The chick B cells maintained a high intracellular taurine concentration (0.8–1.12 m*M*) that decreased with age. The B cells exhibited carrier-mediated and simple diffusion uptake components, but only the carrier-mediated component increased with age. Inhibitor studies indicated taurine uptake was sodium and energy dependent. The data demonstrate that chick B cells possess a specialized taurine transport system and the activity of this system changes during posthatch B cell development.**
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The chicken B cell is an important component of the chicken immune system (1). The biological activity of the chicken B cell steadily increases after hatch (2), and by 4 weeks of age, the mature chicken B cell immune system is considered to be fully functional (3–5). Several factors may be involved in the process that results in full immunocompetence (2), one of which may be taurine.

At Day 12 of embryonic development, cells in the bursa begin to proliferate, and this proliferation continues until 2–4 weeks after hatching (3–5). A previous study demonstrated that taurine (2-aminoethanesulfonic acid) is required for the optimal proliferation rate by human lymphoblastoid cells (6), a function which may also exist in chick B cells. Also, another study has suggested that taurine plays a role in bursa gland development (7). Hence, taurine may be involved in bursa gland development and B cell proliferation.

Studies in the chicken have shown that the plasma taurine concentration significantly increases during the first 2 weeks after hatching and averaged 104 μM from Weeks 2–6 (8). The ability of two leukocyte populations to actively absorb taurine and maintain the cell to plasma taurine gradient also significantly increased during the same period after hatching (8). Thus, the increase in chick B cell biological activity after hatching (2) may involve the ability of chick B cells to actively absorb taurine during this early developmental period.

The present study was designed to determine (i) the taurine content in the Bursa of Fabricius and B cells during the first 6 weeks after hatching and (ii) the kinetic properties of the B cell taurine uptake system with age.

Materials and Methods

Maintenance of Animals. Broiler strain chickens were hatched and maintained at the West Virginia University Poultry Farm (Morgantown, WV). The chickens were housed in floor pens and had access to diet and water *ad libitum*. The diet was a standard commercial corn-soybean meal ration adequate in all nutrients.

B Cell Isolation. Chickens were sacrificed using chloroform and the bursa glands were removed at weekly intervals after hatching. The bursa glands were washed with modified Hanks' balanced salt solution (137 mM NaCl, 5.4 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, and 5.55 mM glucose [pH 7.4]) and then placed in a plastic petri dish (100 × 15 mm) with approximately 4 ml of Hanks' balanced salt solution. Approximately 1–3 g of bursa gland were used for each isolation. Therefore, the number of bursa glands required at each age differed as the bursa gland weight increased with age. The bursa were teased apart with forceps and this mixture was filtered through cheesecloth. The petri dish was washed with an additional 4 ml of Hanks' balanced salt solution and filtered. To obtain the last B cells, the cheesecloth filter was manually squeezed and this solution was added to the filtrate. The filtrate (8.0 ml) was then layered on 3.0 ml of Histopaque-1077 (Sigma) in a 15-ml conical centri-

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fuge tube and centrifuged at 400*g* for 30 min. The B cell layer was carefully removed at the buffer/Histopaque-1077 interface, resuspended in 8.0 ml of HEPES buffer (10 m*M* *N*-(2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid, 145 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, and 5.5 m*M* glucose [pH 7.4]), and centrifuged at 400*g* for 5 min. The supernatant was removed and the B cells were washed twice in HEPES buffer.

The B cells were counted on a Coulter Counter model Zm. The B cell size distribution, mean cell volume, and purity were determined using a Coulter Channelyzer interfaced with the Coulter Counter Zm. The intracellular water content of B cells was conducted as described previously (8).

Taurine Determination. The taurine content of the bursa gland and the B cells was determined by high performance liquid chromatography (9). Bursa gland tissue (0.1–0.3 g) was homogenized in 2.0 ml of homogenization buffer (10 m*M* *N*-(2-hydroxyethyl) piperazine-*N'*-2-ethanesulfonic acid [pH 7.4]) using a Potter Elvehjem-style tissue homogenizer with a teflon pestel, followed by deproteinization in a boiling water bath for 15 min. The denatured protein and cell debris were removed by centrifugation at 900*g* for 10 min. Isolated B cells (10×10^6 cells) were deproteinized by boiling for 15 min and centrifugation at 14,000*g* for 5 min.

Taurine Uptake Time Course and Inhibitor Studies. An aliquot of B cells (2×10^6 cells) was preincubated at 41°C for 30 min in 400 μl of taurine uptake buffer (10 m*M* HEPES, 145 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, 5.5 m*M* glucose, and 100 μ*M* taurine [pH = 7.4]). At the end of the preincubation period, 100 μl of taurine uptake buffer with 0.05 μCi of [1,2-^{14}C]taurine (New England Nuclear) was added to initiate the uptake assay. The cells were incubated from 10 to 180 min and the uptake was terminated by the addition of 0.5 ml of ice-cold HEPES buffer and pelleted by centrifugation (14,000*g* for 1 min). The pellets were washed twice by alternate resuspension in 0.5 ml of ice-cold HEPES buffer followed by centrifugation (14,000*g* for 1 min). After the final wash, 25 μl of Soluene 350 (Packard Instrument Co.) were added to each pellet for digestion (1 hr at 55°C). The digested cells were added to 10 ml of Scintiverse II (Fischer Scientific) and the ^{14}C activity was measured in a Beckman LS-1800 scintillation counter.

The taurine uptake assay was modified to assess the effects of metabolic inhibitors and sodium on taurine uptake. The B cells were prepared for taurine uptake as described above, except the buffer contained an inhibitor (1 m*M* ouabain, 100 μ*M* cysteine sulfinic acid, 100 μ*M* β-alanine, and 1 m*M* dinitrophenol) or had a decreased sodium concentration (72.5 m*M*). The assay was conducted for 30 min and terminated as described above.

Concentration Dependence and Kinetic Parameters of Taurine Uptake. The B cells (2×10^6 cells) were pelleted by centrifugation at 14,000*g* for 1 min and resuspended in 495 μl of HEPES buffer with taurine concentrations that ranged from 10 to 500 μ*M*. The cells were preincubated for 30 min at 41°C in the HEPES buffer with taurine concentrations from 10 to 500 μ*M*, since these taurine concentrations were selected for the kinetic studies. The uptake assay was initiated by adding 5 μl of HEPES buffer with 0.05 μCi of [1,2-^{14}C]taurine. The assay was conducted for 30 min and stopped with the addition of 0.5 ml of ice-cold HEPES buffer. The pellets were washed twice by alternative resuspension in 0.5 ml of ice-cold HEPES buffer, followed by centrifugation at 14,000*g* for 1 min. The cells were digested and counted as described previously.

The taurine transport protein kinetic parameters K_m and V_{max} were determined from a double reciprocal plot of the total flux. Using a mathematical curve-stripping technique, the total flux were separated into carrier-mediated and simple diffusion components (10).

Statistical Analysis. The B cells were isolated from pooled bursa gland samples during two trials. Data were analyzed by analysis of variance for a completely randomized design, and means were compared by one degree of freedom comparisons.

Results

The cell-size distribution of isolated B cells showed a single major cell population between 4 and 8 μm that did not change with age. The mean cell volume and purity did not differ significantly with age ($P > 0.05$) and averaged 171 μm^3 and 86.2%, respectively. The B cell intracellular water did not differ significantly with age ($P > 0.05$) and averaged 0.32 ± 0.06 μl of $H_2O/10^6$ cells.

The taurine content of the bursa gland (Table I) decreased 46% from 3.94 ± 0.61 (Weeks 1 and 2) to 2.11 ± 0.16 mmol taurine/g bursa gland tissue (Weeks 3 through 6). The B cell taurine concentration (Table I) decreased 29% from 1.14 ± 0.09 m*M* (Weeks 1 and

Table I. Bursa Taurine Content and B Cell Intracellular Taurine Concentration[a]

Age (weeks)	Bursa taurine[b] (mmol/g)	B Cell taurine[c] (m*M*)
1	4.07 ± 0.58	1.23 ± 0.08
2	3.80 ± 0.63	1.04 ± 0.10
3	2.21 ± 0.22	0.75 ± 0.04
4	2.08 ± 0.13	0.80 ± 0.06
5	2.00 ± 0.18	0.77 ± 0.06
6	2.13 ± 0.10	0.93 ± 0.10

[a] Values represent mean ± SE.
[b] Tissue wet weight was used. $n = 3$ at all ages. Weeks 1 and 2 are significantly higher ($P < 0.05$) than weeks 3 through 6.
[c] Intracellular water: 0.32 ± 0.06 μl/10^6 cells used for calculations. $n = 6$ at all ages. Weeks 1 and 2 are significantly higher ($P < 0.05$) than weeks 3 through 6.

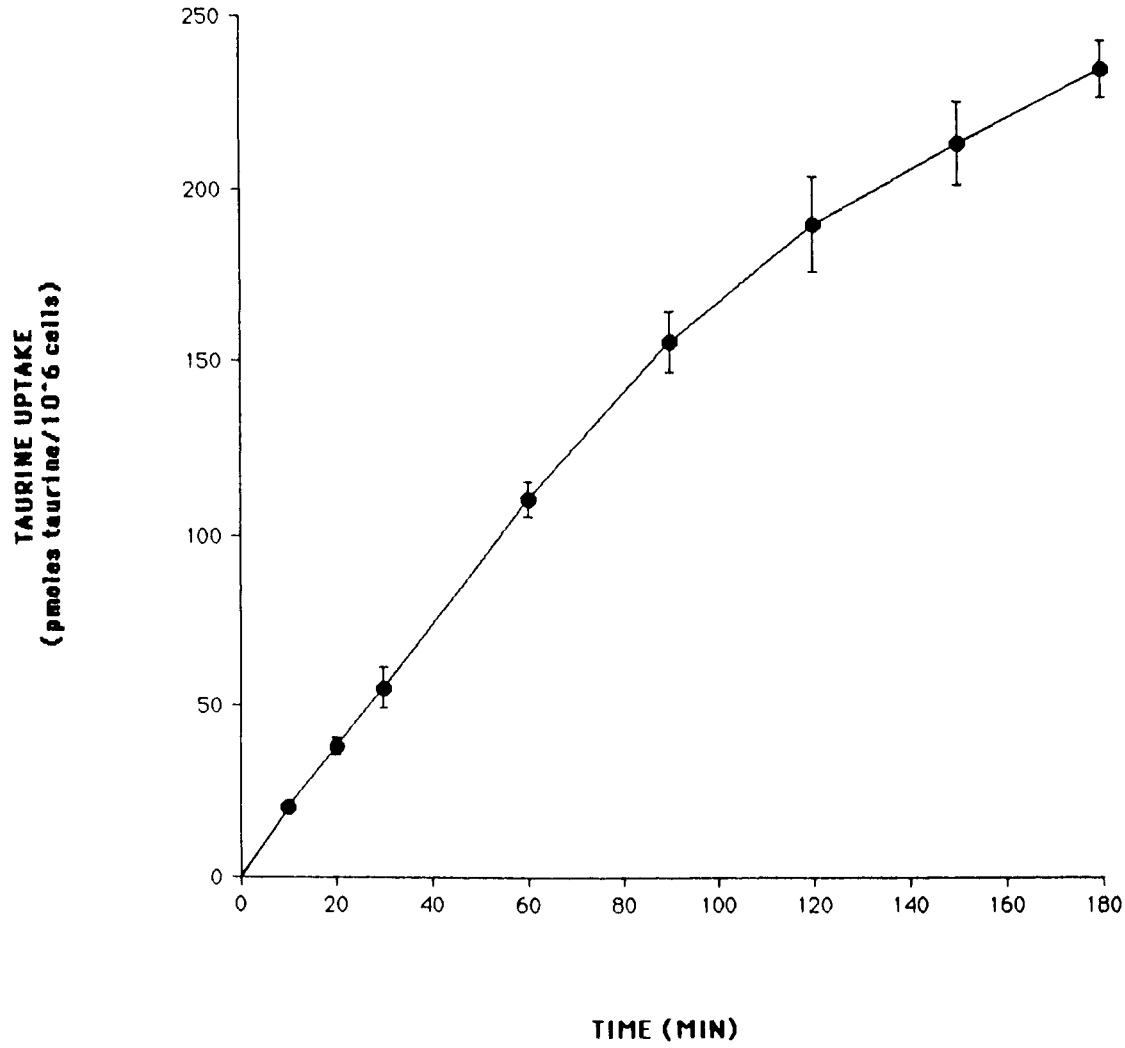

Figure 1. Representative time course of taurine uptake by chicken B cells. Values are mean ± SE for four experiments. (Experimental data from B cells isolated from 5-week-old chickens.)

2) to 0.81 ± 0.07 mM (Weeks 3 through 6). Thus, while the taurine content of the bursa gland and B cell decreased at the same age, the degree of the decrease was greater for the bursa gland.

Taurine uptake by B cells over a 3 hr *in vitro* experiment was curvilinear (Fig. 1). The taurine uptake was linear over the initial 30 min; therefore, the uptake rate was determined using data at 20 min.

Table II shows that the K_m value did not differ significantly with age ($P > 0.05$) and averaged 36.0 ± 9.0 μM. The V_{max} value significantly increased ($P > 0.05$) from 1.45 ± 0.11 (Weeks 1 and 2) to 2.31 ± 0.26 pmol taurine/10^6 cells/min (Weeks 3 through 6). This represents a 59% increase in the rate of B cell taurine uptake. The taurine uptake rate (Table II), which was determined from the linear component of the taurine uptake time course, significantly increased ($P < 0.05$) from 1.32 ± 0.12 (weeks 1 and 2) to 2.11 ± 0.16 pmol taurine/10^6 cells/min (Weeks 3 through 6). This represents a 63% increase in the rate of taurine uptake. The V_{max} values and uptake rate at each age were similar. Additionally, the increase in the rate of taurine uptake was concurrent with the observed decrease in the bursa gland and the B cell taurine content (Table I).

Table II. Effect of Age on B Cell Taurine Uptake Kinetic Parameters[a]

Age (weeks)	K_m[b] (μM)	V_{max}[c]	Uptake rate[d]
1	40.2 ± 10.6	1.36 ± 0.11	1.12 ± 0.13
2	34.4 ± 10.5	1.54 ± 0.10	1.51 ± 0.11
3	34.7 ± 15.7	2.39 ± 0.60	2.01 ± 0.21
4	32.3 ± 4.3	2.30 ± 0.14	2.33 ± 0.25
5	43.2 ± 9.0	2.33 ± 0.18	1.98 ± 0.06
6	33.4 ± 3.9	2.24 ± 0.13	2.10 ± 0.12

[a] Values represent mean ± SE. $n = 4$ at all ages.
[b] No significant difference ($p > 0.05$) with age.
[c] Weeks 1 and 2 are significantly lower ($P < 0.05$) than weeks 3 through 6. V_{max}: pmol taurine/10^6 cells/min.
[d] $n = 4$ at all ages. Weeks 1 and 2 are significantly lower ($P < 0.05$) than weeks 3 through 6. Uptake rate determined at 20 min in time course experiment. Uptake rate: pmol taurine/10^6 cells/min.

The B cell taurine uptake rate was affected by the extracellular taurine concentration, which indicated that the total taurine flux consisted of two processes, carrier-mediated uptake and diffusion (Fig. 2). Analysis of the concentration dependence data allowed the two components of the taurine transport system to be quantified (Table III). The total flux increased significantly ($P < 0.05$) from 1.23 ± 0.06 (Weeks 1 and 2) to 1.72 ± 0.24 pmol taurine/10^6 cells/min (Weeks 3 through 6). The carrier-mediated component of the total flux significantly increased from 0.84 ± 0.08 (Weeks 1 and 2) to 1.43 ± 0.22 pmol taurine/10^6 cells/min (Weeks 3 through 6), whereas the simple diffusion component did not differ significantly with age ($P > 0.05$) and averaged 0.32 ± 0.08 pmol taurine/10^6 cells/min. Thus, the increase in the B cell taurine flux with age is due to an increase in the carrier-mediated component of the taurine transport system. This component comprises from 68% (Weeks 1 and 2) to 83% (Weeks 3 through 6) of the total taurine flux.

Further characterization of the B cell taurine transport system was accomplished by the use of inhibitors (Table IV), which have previously been shown to affect taurine transport (10). A 50% decrease in extracellular

Table III. Effect of Age on Components of the B Cell Taurine Uptake System[a]

Age (weeks)	Total taurine flux[b]	Component	
		Carrier-mediated[b]	Simple diffusion[c]
1	1.20 ± 0.07	0.73 ± 0.09	0.47 ± 0.06
2	1.26 ± 0.04	0.94 ± 0.07	0.32 ± 0.10
3	1.60 ± 0.32	1.28 ± 0.27	0.32 ± 0.11
4	1.92 ± 0.33	1.70 ± 0.30	0.22 ± 0.04
5	1.63 ± 0.19	1.35 ± 0.15	0.28 ± 0.05
6	1.71 ± 0.11	1.39 ± 0.14	0.32 ± 0.09

[a] Values represent mean ± SE. values represent pmol taurine/10^6 cells/min calculated for taurine concentration of 100 μM. $n = 4$ at all ages.
[b] Weeks 1 and 2 are significantly lower ($P < 0.05$) than weeks 3 through 6.
[c] No significant difference ($P > 0.05$) with age.

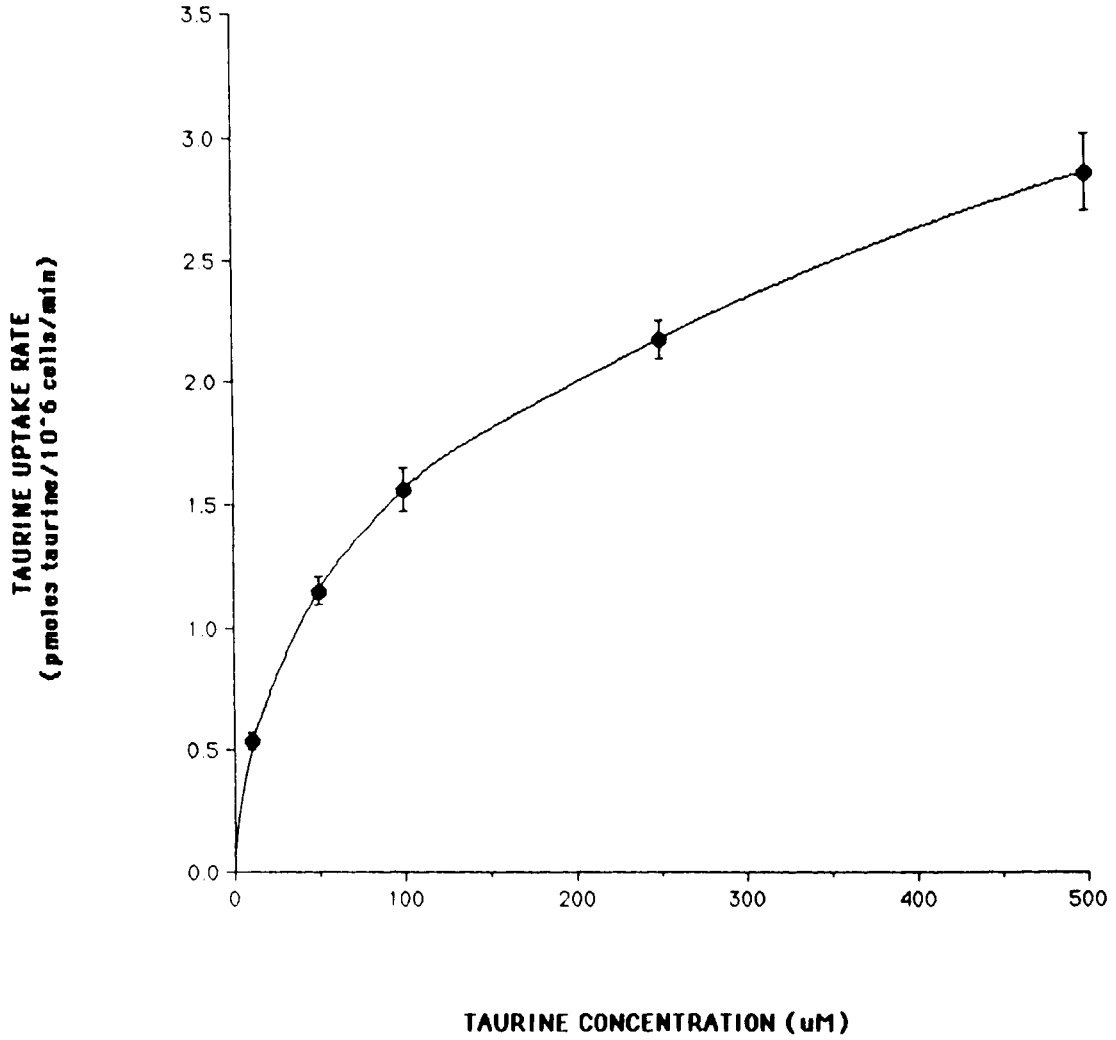

Figure 2. Representative taurine uptake as a function of extracellular taurine. Values are mean ± SE for four experiments. (Experimental data from B cells isolated from 6-week-old chickens.)

Table IV. Effect of Inhibitors on B Cell Taurine Uptake with Age[a]

Age (weeks)	Low Na^+ (72.50 mM)	Ouabain (1 mM)	CSA (100 μM)	B-alanine (100 μM)	Dinitrophenol (1 mM)
2	36.0 ± 2.6	72.6 ± 8.4	84.8 ± 5.4	65.3 ± 4.6	46.5 ± 4.5
4	43.3 ± 6.3	70.8 ± 5.4	76.6 ± 5.0	67.3 ± 4.7	47.7 ± 9.7
6	44.2 ± 6.2	66.4 ± 2.1	90.8 ± 3.7	79.2 ± 7.8	49.1 ± 1.9

[a] Values represent mean ± SE ($n = 4$). Values expressed as percentage of the control value. There was no significant difference with age ($P > 0.05$) for any of the inhibitors.

sodium reduced taurine uptake to 41.2% of control value, while the Na^+,K^+-ATPase inhibitor ouabain decreased taurine uptake to 69.6% of control value. In the presence of the competitive inhibitors of the β-amino acid transport system, taurine uptake was reduced 20% by equimolar β-alanine and 16% by equimolar cysteine sulfinic acid. The energy dependence of B cell taurine uptake was shown with the uncoupling agent dinitrophenol which decreased taurine uptake to 47.8% of control values. There was no significant age effect ($P > 0.05$) for any of the inhibitors used.

Discussion

The cell diameter distribution of isolated B cells showed a single major peak, with cell diameters from 4 to 8 μm. This cell diameter range is in agreement with that reported previously for chicken B cells (11). On this basis, we concluded that the major cell population isolated from the bursa was composed of B cells and the purity was high (86.2%) each week and did not change with age.

The plasma taurine concentration during the first 14 days after hatching increased and became constant at 104 μM from Weeks 3 through 6 (8). The bursa has an extensive vasculature (12); thus, the bursa gland is exposed to the increasing plasma taurine concentration. In contrast to the increase in plasma taurine concentration with age, the bursa taurine content significantly decreased at Weeks 3 through 6 (Table I). This suggests that the decrease in bursa gland taurine content is not due to an inadequate supply of taurine from the plasma, because the bursa gland taurine content decreased when the plasma taurine concentration had just reached its adult physiological concentration. Therefore, the significant decrease in bursa gland taurine content may be related to the developmental process, which continues in the early posthatch period (3, 5, 13).

Exposure of the B cells to the plasma will depend on the location of the B cell within the gland, since the cortex has more vasculature than the medulla (14). Consequently, B cell intracellular taurine concentration relative to the plasma taurine concentration was not considered, since the B cells were harvested from the entire gland.

The K_m of B cell taurine transport did not differ significantly with age and averaged 36 μM (Table II), suggesting that the increased efficiency or number of taurine transport proteins, rather than the induction of an isotransport protein, accounts for the increased taurine uptake at Weeks 3 through 6. The inhibitor study results also support this interpretation. If differential expression of an isotransport protein had occurred, the effects of the inhibitors would probably differ with age, especially the β-amino acid transport system competitive inhibitors β-alanine and cysteine sulfinic acid. However, the percentage of inhibition of these inhibitors did not significantly differ with age (Table IV).

However, the V_{max} of taurine uptake significantly increased at Weeks 3 through 6 (Table II), which suggests that the increased taurine uptake rate was due to an increased number of taurine transport proteins per cell. This is consistent with the determination that the carrier-mediated component of taurine uptake increased at Weeks 3 through 6 (Table III).

The increase in the taurine uptake rate at Weeks 3 through 6 (Table II) would be expected to result in a simultaneous increase in the B cell intracellular taurine concentration. However, the B cell intracellular taurine concentration significantly decreased during this period (Table I). This unexpected finding suggested that the free intracellular taurine pool, which is maintained by the uptake of extracellular taurine (15), had increased at Weeks 3 through 6 even though the total intracellular taurine content had decreased. This would account for the increase in the taurine uptake rate, because it is required to maintain the larger free intracellular taurine pool. This is consistent with the finding that the bursa taurine content is significantly lower at Weeks 3 through 6 (Table I).

The inhibitor studies (Table IV) indicated that taurine uptake by chicken B cells is energy dependent and has specificity for β-amino acids, and that lowered extracellular sodium decreased taurine uptake. The decreased B cell taurine uptake in low extracellular sodium was viewed as a result of decreased taurine uptake via the Na^+ cotransporter. However, it has been reported that decreased extracellular sodium caused increased taurine efflux in a variety of mammalian cells (16, 17) and pigeon erythrocytes (18) due to decreased osmolarity. The osmolarity of the low Na^+ buffer was not adjusted to that of the control buffer; thus, the increased taurine efflux may have contributed to the decreased taurine uptake in the low Na^+ buffer.

The hypothesis that animal cells accumulate inorganic ions and maintain gradients as a means of cellular regulation has been well documented. These studies showed that at the earliest age at which B cell harvest is possible, the taurine gradient and active transport were functional. Taurine, a zwitterion at physiological pH, is an organic ion that has been described as a cell regulator (15). One such regulatory role of taurine is its requirement for the optimal proliferation rate of human lymphoblastoid cells (6), a function that may also exist in chicken B cells. The process by which taurine may effect chicken B cell proliferation has not been determined, but it may involve the regulation of calmodulin and cellular calcium (19), since both are known elements in the control of cell division (20).

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