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# Immunohistochemical localization of taurine in various tissues of the mouse

**A. Terauchi**[1,2], **A. Nakazaw**[1], **K. Johkura**[1], **L. Yan**[1], and **N. Usuda**[1]

[1] Department of Anatomy and Cell Biology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan
[2] Department of Pediatrics, Inariyama Medical and Welfare Center, Kohshoku, Nagano, Japan

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**Summary.** The localization of taurine was investigated in several tissues of the mouse. Immunohistochemical methods using a polyclonal antibody for taurine derived from rabbits was used in these studies. This method was used since it is a simple procedure and the results are clear and reliable. Tissues were fixed with paraformaldehyde, embedded in paraffin and treated in a microwave oven before using an avidin-biotin-complex method (ABC method). Control staining was accomplished by employing absorption staining using various amino acids: taurine, arginine, cysteine, hypotaurine and others. For purposes of comparison, radioautography (RAG) with $^{3}$H-taurine was performed to confirm the reliability of the immunohistochemical staining compared with the localization of the $^{3}$H-taurine incorporation in endothelial cells of the blood vessels of several tissues. In this investigation, immunoreactivity was broadly observed in many tissues: Purkinje cells of the cerebellum, glia cells of brain tissue, cardiac muscle cells, matrices of the bone, mucus granules of goblet cells of the intestines, and brown adipose cells of the fetus. Although the meaning of this widespread localization of taurine can not be explained completely, we surmise that taurine may have a different function in each of the tissues. In addition, taurine reactivity was observed in cell nuclei which was evidence of the presence of taurine in the nuclei.

**Keywords:** Amino acids – Taurine – Taurine-antibody – $^{3}$H-Taurine

## Introduction

The biological effects of taurine have been widely investigated. Taurine protects hepatic cells from the toxic action of nitric oxide (Redmond et al., 1996), has the role of osmoregulator in neural tissues (Menendez et al., 1990), and controls the duration of the action potential of ventricular papillary muscles (Lake et al., 1990). In the last 10 years, immunohistochemical staining has

been used for the purpose of observing the localization of taurine (Ding et al., 1993; Decavel et al., 1995). However, the methods have differed among the various investigators and there has not been complete agreement concerning the results. In this study, we tested several tissues systematically using a single method. Our results support the reports of other investigators on nervous tissue (Shupliakov et al., 1994) and renal tissue (Ma et al., 1994).

## Materials and methods

### *Radioautography*

[In vivo RAG]: One ml of $^{3}$H-taurine solution (37 MBq/ml) was injected into the peritoneum of a mouse and after 60 minutes, several organs were removed. [*In vitro* RAG]: Brain, heart, liver, kidneys, and skeletal muscles were cut into pieces and incubated for 5, 15, 30 and 60 minutes in a culture medium containing 3.7 MBq/ml of $^{3}$H-taurine. For the control experiments, $^{3}$H-arginine and $^{14}$C-cysteine were employed instead of $^{3}$H-taurine.

The tissues in both methods were fixed with 2.5% glutaraldehyde and 1% osmium tetroxide, dehydrated with ethanol and embedded in Epon. The specimens were cut into approximately 1 μm sections, mounted, and then treated with NR-M2 emulsions for light microscopic radioautography. After exposure from 2 to 24 months, the specimens were developed in SDX-1, stained with toluidine blue, and observed using light microscopy and electron mycroscopy.

### *Immunohistochemical staining*

Organs of a 15-day-old fetal mouse and 21-week-old adult mouse were fixed with a 4% paraformaldehyde solution. The tissues were rinsed with phosphate buffered saline, dehydrated in ethanol, embedded in paraffin, and cut into 5 $\mu m^3$ pieces. The sections were mounted on glass slides, deparaffinized in xylene, dehydrated in ethanol, and boiled for 5 minutes in a microwave oven for antigen retrieval. Thereafter, 1.5% normal goat serum was applied on the sections for the purpose of removing unspecific antigen and left for 60 minutes in a moisture chamber. The goat serum was then shaken off and anti-taurine antibody was applied, and left for 16 hrs in a moist chamber in a cold room. The antibody against taurine was diluted in 1.5% normal goat serum, 1:900, 1,800 and 5,400, for purposes of comparison to judge which concentration was optimal. The sections were then treated with 1:200 biotinized rabbit IgG produced in a goat and left for 60 minutes, next treated with 1:4,000 avidin-biotin-complex and left for 60 minutes at room temperature, and finally treated with 0.02% 3.3-diaminobenzidine 4HCl solution containing 0.0045% $H_2O_2$ and left for 10 minutes. Sections were dehydrated in propylalcohol, and immersed in xylene and mounted. The control sections were treated using the same procedure except that they were incubated with the antibody against taurine. The anti-taurine antibody was purchased from Serotec Co. (Oxford, England).

### *Examination of specificity with preabsorption test*

In order to investigate the specificity of the taurine antibody, tissues were stained with the taurine antibody after pretreatment with various amino acids: 200 μg/ml of taurine, hypotaurine, aspartic acid, proline, glycine, homocysteine, cysteine, arginine and GABA.

## Results

### *Radioautography*

$^{3}$H-Taurine was heavily incorporated into the nuclei and cytoplasm of the endothelial cells of the blood vessels in the brain and skeletal muscle (Fig. 1a), epithelial cells of the intestines, sheath of the peripheral nerve, epithelial cells of the renal tubules, and Bowman's capsular cells. On the other hand, $^{3}$H-arginine and $^{14}$C-cysteine were incorporated only slightly into the blood vessels. Incorporation of $^{3}$H-taurine was also observed in the nuclei and nucleolus of endothelial cells of the capillary (Fig. 1b) and in skeletal muscle cells.

### *Immunohistochemical staining*

Immunoreactivity was indicated by a brown color and differed in intensity of color by several degrees. Control sections were observed translucently. The contrast in staining was optimal when the taurine antibody was diluted 1:1,800. In the absorption staining process, the tissues which were applied with taurine-antibody and pretreated with taurine showed a reduced intensity of stain compared with the tissues pretreated with other amino acids. The nuclei and cytoplasm of the endothelial cells of the capillaries, the venules and the arterioles were stained intensely (Fig. 2) in every tissue. The nuclei of

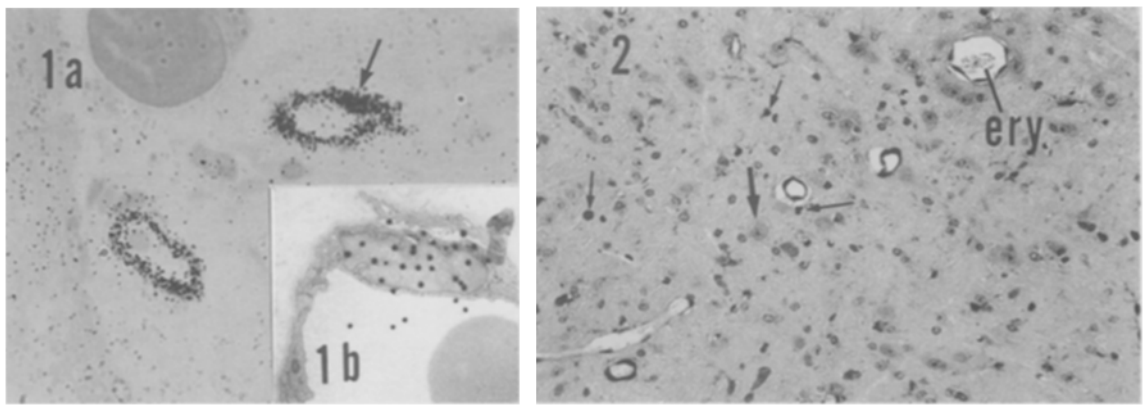


**Fig. 1. a** Light-microscopic radioautograph of mosue skeletal muscle. Two capillaries heavily labeled with $^{3}$H-taurine are observed. $^{3}$H-Taurine is taken up into both nuclei (arrow) and cytoplasm. Section (1 μm) were embedded in Epon and stained with toluidine blue. (×1,800). **b** Electron-microscopic radioautograph of capillary of the same specimen. Uptake of $^{3}$H-taurine is observed in the nuclei of the endothelial cell. (×9,100)

**Fig. 2.** Cerebral tissue. Several blood capillaries and small glia cells (thin arrows) are stained intensely, neural cells (large arrows) are stained moderately and axon and matrix are stained faintly. Erythrocytes were very faintly stained (*ery*). (×180) For Fig. 2 through 8 the tissues were embedded in paraffin and stained according to the ABC method after treatment in the microwave. Light-microscopic photographs of the tissues were taken after staining with the polyclonal antibody to taurine. See Materials and methods section for details

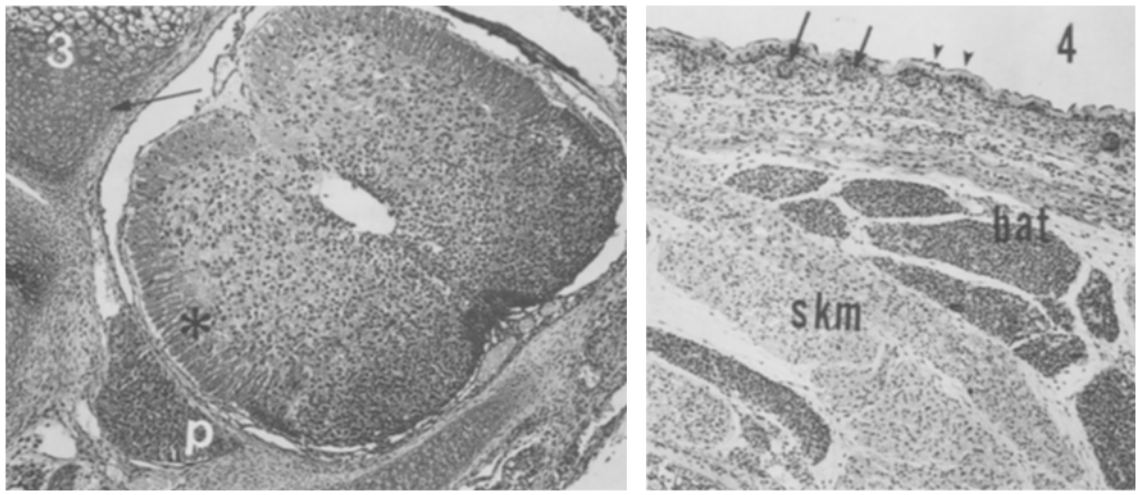


**Fig. 3.** Cross section of the spinal cord at the level of upper thorax. Neural cells and axons (large asterisk) in the spinal cord, cells of paravertebral ganglion (*p*) and matrices of the bones (arrow) are stained moderately. (×178)

**Fig. 4.** Surface of the cross section of the 15-day-old fetus. Epidermis (arrow heads), hair folicles (arrows) and brown adipose tissues (*bat*) are intensely stained. Skeletal muscle tissues (*skm*) are moderately stained. (×180)

all the tissues were stained moderately; on the other hand, the erythrocytes were weakly stained. In the 15-day-old fetus, the ependymal cells, the glia cells and Purkinje cells of the cerebrum and cerebellum, neural cells of paraganglia (Fig. 3), and axons and cell bodies in the vertebra were stained. In the intestines, nuclei of the epithelial cells in the mitotic phase were stained intensely and mucous granules of the goblet cells were stained moderately. Matrices of the bone, the cells of the dermis, and brown adipose cells were moderately stained (Fig. 4). In the adult mouse, glia cells, ependymal epithelial cells, Purkinje cells (Fig. 5) were stained intensely or moderately. In the kidney, epithelial cells of distal and collecting tubules, Bowman's capsular cells, and basement membrane of the tubules in the renal medulla were intensely stained (Figs. 6 and 7). In the digestive organs, gastric mucosal cells, epithelial cells and mucosal granules of the goblet cells of the intestines were moderately stained (Fig. 8). The mucous on the surface of the stomach was faintly stained. When reactivity was compared among the various muscle tissues, cardiac muscle was stained intensely while smooth muscle was stained faintly. Skeletal muscle was stained intermediately. Localization of immunolabeling of these tissues is summarized in Table 1.

## Discussion

Antiserum of taurine have been made by several investigators from rabbits (Tomida et al., 1985; Yoshida et al., 1986). Thereafter, many observations using taurine-antibody have been reported. In this report, immunoreaction

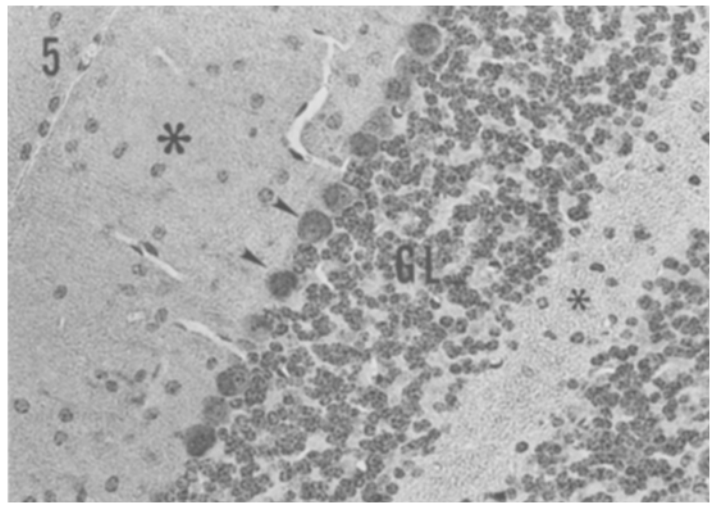


**Fig. 5.** Cerebellum of the adult mouse. Purkinje cell somata (arrowheads) and nuclei of the cells in the granular layer (*GL*) are darkly stained. Matrices of the molecular layer (large asterisk) and medulla (small asterisk) are faintly stained. (×720)

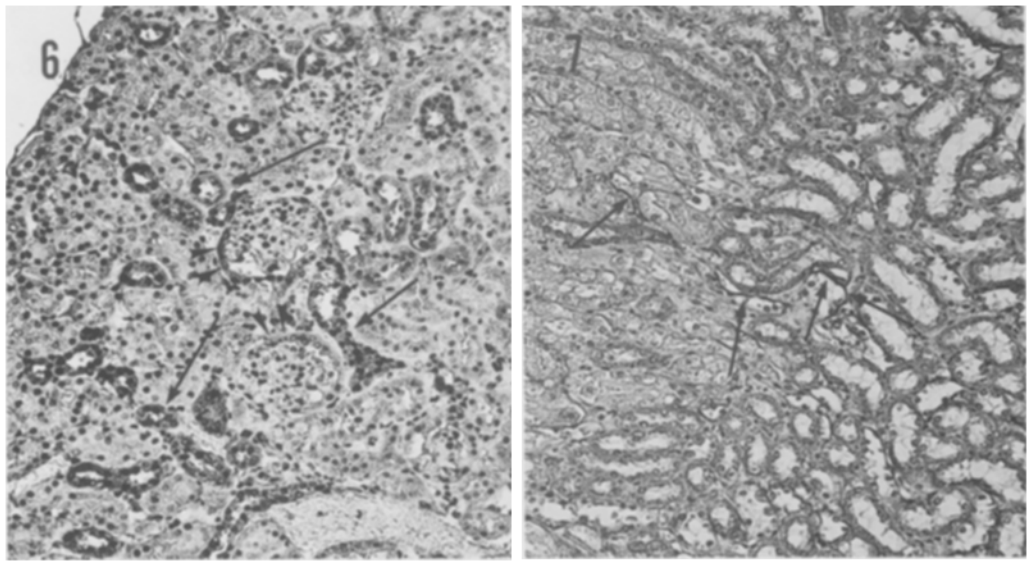


**Fig. 6.** In the renal tissue of the adult mouse, distal tubules (arrows) and Bowman's capsular cells (arrow head) are intensely stained. (×360)

**Fig. 7.** Renal medulla of the same specimen (Fig. 6). Basement membrane is intensely stained (arrows). (×360)

against taurine was broadly observed in tissues and cells in intensity of varying degrees. In these results, the presence of immunoreactivity in the brain and kidney agrees with the results of other investigators (Ida et al., 1987; Tomida and Kimura, 1987; Ma et al., 1994; Gragera et al., 1994). However, the presence of taurine such as in cell nuclei, ependymal cells of brain, epithelial cells

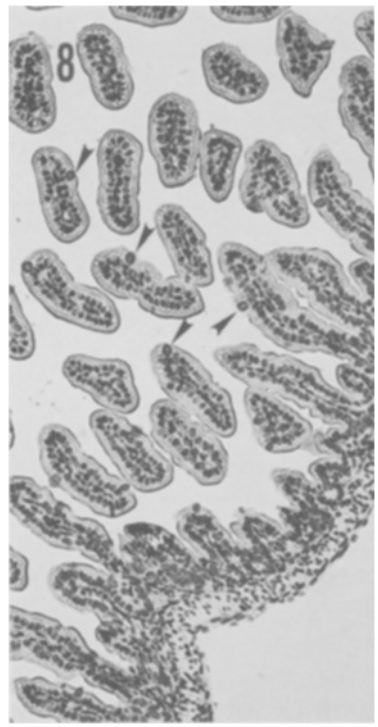

**Fig. 8.** Intestine of the adult mouse. Cell nuclei and mucous of the goblet cells (arrow heads) are darkly stained. (×180)

of epidermis, mucous granules of the goblet cells, matrix of the bone, and brown adipose tissue has not been explained.

It has previously been reported that glial cells, Purkinje cells, ependymal cells, and astrocytes of nervous tissue are intensely stained. On the other hand, axons exhibit low reactivity (Tomida and Kimura, 1987, Lee et al., 1992, Shupliakov et al., 1994). Our staining pattern was similar. Moreover, we observed that fetal neural axons were stained more intensely than adult axons. During differentiation, taurine facilitates the movement of certain neural substances along the axon. Although, taurine is found in a high concentration in nervous tissues, the incorporation of $^{35}$S-taurine into the brain proceeds slowly in comparison to other organs. In agreement with Iwata et al. (1978) we found that considerable amounts of $^{3}$H-labeled taurine were detected in the epithelial cells of the renal tubules, intestines, and skeletal muscle 60 minutes after the injection of taurine. However, at the same time, very little radioactive taurine was found in the brain and heart, with the expection of the capillary blood vessels and glial cells. This result suggests that administered taurine is first taken up by blood vessels and the glial cells near the capillary, and then slowly accumulated in the brain.

The histochemical technique also detected taurine in the cell nuclei. However, the method may include nonspecific reactions between the taurine-antibody and amino acids other than taurine. Concerning this point, the cross-reactivity of taurine antiserum with other amino acids is very low, estimated to

**Table 1.** Summary of immunohistochemical localization of taurine

| | Tissue / cell | Intensity of immunoreactivity | | | |
|---|---|---|---|---|---|
| | | strong | moderate | weak | faint or no reaction |
| general | endothelial cells of the blood vessels | * | | | |
| | cell nuclei | | * | | |
| | erythrocytes | | | | * |
| | white blood cells | | * | | |
| | platelets | | * | | |
| | cytoplasm of the white adipose cells | | | | * |
| cerebrum | glia cells | * | | | |
| | neuronal cells | | * | | |
| | axon | | | * | |
| | matrices of the | | | | |
| | granular layer | | * | | |
| | nuclei of the cerebrum | | * | | |
| | axonal layer | | | * | |
| | ependimal cells | | * | | |
| cerebellum | glia cells | * | | | |
| | Purkinje cells | | * | | |
| | axons | | | * | |
| | matrix of the | | | | |
| | molecular layer | | | * | |
| | granular layer | | * | | |
| | white matter | | | * | |
| kidney | distal tubules | * | | | |
| | basement membrane of tubules | * | | | |
| | Bowman's capsular cells | * | | | |
| muscular tissues | smooth muscle | | | * | |
| | cardiac muscle | | * | | |
| | skeletal muscle | | | * | |
| 15-day-fetus | | | | | |
| CNS | glia cells | * | | | |
| | axons | | * | | |
| | paravertebral ganglia cells | | * | | |
| others | matrix of bone | | * | | |
| | brown adipose tissue | | * | | |
| | granules of the goblet cells | | * | | |
| | mitotic nuclei in the intestine | * | | | |

be less than 0.5% (Ida et al., 1987). The existence of taurine in cell nuclei has been observed either immunohistochemically or by autoradiography in glial cells of the optic nerve (Decavel et al., 1995), in endothelial cells of blood capillaries (Terauchi et al., 1993) and in bullfrog sympathetic ganglia cells (Sakai et al., 1989). Additionally, taurine has been reported to modulate $Ca^{2+}$ and $Na^{+}$ transport into cell nuclei (Bkaily et al., 1997). From these results, we surmise that taurine is taken up into the cell nucleus, where it accumulates. However, the role of taurine in the nucleus remains unclear.

In several tissues, the role of taurine cannot be explained. Matrices of the bone, mucous granules of the epithelium of goblet cells and renal tubular basement membrane all showed immunoreactivity. These tissues contain collagen, glycoprotein or keratin with high levels of sulfur. In bacteria, taurine utilization as a source of sulfur is controlled genetically (Ploeg et al., 1996). It is thought that taurine serves as an important source of sulfur. White adipocytes do not exhibit immunoreactivity, but brown adipocytes exhibit intense staining. Brown adipocytes are rich in mitochondria for energy production and the metabolism of fatty acids (Garlid et al., 1996). Regarding the relationship between taurine and mitochondria, Lombardini et al. (1994) recently reported that taurine inhibits the phosphorylation of specific proteins in heart mitochondria. The immunolabeling pattern of brown adipocytes supports a role for taurine in the mitochondria.

Erythrocytes were shown to be only faintly stained. In a previous radioautographic study, $^{3}$H-taurine was found not to be incorporated into erythrocytes (Terauchi et al., 1993). Using biochemical assays, red blood cells were found to contain taurine but the level is much lower than that found in granule cells or platelets (Laidlow et al., 1987). Another study concluded that taurine's effect on the erythrocyte membrane protected the cell from hyperosmotic stress (Nakamua et al., 1993). How taurine can be protective despite low levels in the erythrocyte remains unexplained. Radioautographic examination of adult mouse, epidermis, hair follicle sheaths and sebaceous glands revealed massive labeling with $^{3}$H-taurine (Shimada et al., 1993). Our results support this observation as immunoreactivity was apparent in the epidermis and hair follicles of the fetus. Our data support the idea that the taurine histochemical method is useful in defining the morphological distribution of the amino acid.

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**Authors' address:** Akiko Terauchi, Department of Anatomy and Cell Biology, Shinushu University School of Medicine, Matsumoto 390, Nagano, Japan.

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