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Taurine in Hearts and Bodies of Embryonic through Early Postpartum CF_1 Mice[1] (41924)

CHRISTOPHER J. QUILLIGAN,*[,2] FREDERICK K. HILTON,*[,3] AND MARY A. HILTON

*Departments of *Anatomy and Biochemistry, University of Louisville Health Sciences Center, Louisville, Kentucky 40292*

Abstract. The hearts and remaining bodies of embryonic and fetal mice of known gestational age and of neonatal mice up to the age of 8.5 days were freeze-dried, weighed, and analyzed for the amino acid, taurine, by high performance liquid chromatography. Although cardiac taurine is only a small fraction of the taurine in the rest of the body in all animals studied, the concentration of taurine in the heart is similar to that in the rest of the body (40–45 nmole/mg freeze-dried wt) in embryos through Day 14.5 of gestation. Cardiac taurine concentration then begins to exceed that of the remainder of the body which gradually declines throughout the period studied. A doubling of cardiac taurine concentration is seen at birth (Day 19.5) when the cardiac to body taurine ratio rises markedly and is maintained at 2–4 throughout the period of observation. A maximum concentration of cardiac taurine (110 nmole/mg freeze-dried wt) is recorded 2.5 days after birth. The dramatic increase in cardiac taurine concentration at the time of birth follows the reported appearance in neonatal mouse hearts of adult levels of β-adrenergic receptors and the increased work load of the heart.

Taurine is an amino sulfonic acid having a wide distribution in the animal, yet relatively little is known about its function (1). The distribution and concentration of taurine in various species have been described by many researchers (1). The most clearly defined role of taurine is that as a constituent of taurocholate, a bile salt in many lower vertebrates and in mammals (2).

Some investigators suggest that taurine has a role in the maintenance of excitatory activity in muscle and nervous tissue of mammals. It is postulated that, in the brain, taurine may be acting as an inhibitory neurotransmitter or neuromodulator (3, 4), and it has been found in brain in lower than normal concentrations at epileptic foci, both in animal models and in humans (5). Taurine has been shown to affect the structural integrity of the retina in the cat. Kittens fed a taurine-free diet experience retinal degeneration with ensuing blindness (6–8). In the dog heart taurine was reported to counteract the arrhythmias produced by administration of epinephrine and of digitalis glycosides (9). Abnormal electrocardiograms of dogs caused by perfusion with strophanthin-K or K^+-free solutions were corrected by taurine administration (10), although more recent work has failed to show such clear-cut effects of this amino acid (11). Taurine was also shown to potentiate the positive inotropic response to strophanthin-K in the isolated perfused guinea pig auricle (12). These findings and other evidence have lead some researchers to suggest that taurine may be acting to stabilize cellular membranes (13). This idea finds support in work by Kramer *et al.* (14), showing that the presence of taurine protects against the decrease in sarcolemmal ATPase activity and increase in sarcolemmal calcium binding induced by calcium-paradox conditions.

Huxtable and Bressler reported increased concentrations of taurine in the hearts of persons who died of congestive heart failure (15, 16). A similar phenomenon had been demonstrated in dogs with congestive heart failure due to experimentally induced pulmonary stenosis (17).

Taurine makes up as much as 50% of the free amino acid pool in the heart (18), and it is actively, although slowly, transported across cardiac cell membranes (19). Active

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[3] To whom all correspondence should be addressed.

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transport appears to be responsible for the high myocardium:plasma ratio of taurine, which in the mouse heart approaches 200:1 (20, 21). The mouse heart is able to synthesize taurine (22) and, while the pathway is not clear, the quantitative contribution of endogenous synthesis to cardiac taurine is considerable (23).

It has been demonstrated *in vivo* that the β-adrenergic agonist, isoproterenol, can induce cardiac hypertrophy with concomitant elevation of cardiac taurine (24). Increased uptake was demonstrated in isolated heart preparations perfused with isoproterenol and taurine (25). Huxtable and Chubb (25) have demonstrated that taurine transport into the heart is modulated by the level of β-adrenergic activation. They proposed that this transport system could be responsible for the elevated levels of taurine seen in human congestive heart failure, which is characterized by chronic β-adrenergic stimulation.

The possibility of establishing correlations between cardiac taurine profiles and events in development, particularly maturation of the β-adrenergic system, led us to examine these relationships in mice during the embryonic, fetal, and postnatal periods. Very little such information exists for the mammalian heart (2), although Grosso *et al.* (26) have determined the taurine content of fetal mouse hearts at 16–19 days gestation. Also, Gonzales and Awapara (27) have reported values for the taurine content of chick embryos at 3- to 4-day intervals throughout incubation and after hatching. Thus we chose to examine taurine concentrations in the hearts and bodies (less the hearts) of mice between 10.5 days of gestation, or approximately when the spinal ganglia give off sympathetic branches near the heart (28), through 8.5 days postpartum, or some 2 days past the time that Wekstein (29) determined that the sympathetic nervous system is fully functional in the rat. Chen *et al.* (30) have demonstrated significant increases in the density of β-adrenergic receptors in fetal mouse hearts throughout the later third of gestation. High densities of these receptors, exceeding those of adult hearts, were demonstrated in 3-day neonatal hearts, with similar values at 14 days postpartum.

Materials and Methods. *Animals.* Randomly mated albino CF_1 mice (*Mus musculus*) from our own colony were used; they were fed Purina mouse chow and water *ad libitum*. A single stud male mouse was placed in a cage overnight with one to three females which were examined the next morning for evidence of a vaginal plug. The first morning a plug was seen was counted as the first half day of gestation. When the embryos or fetuses reached a selected gestational day, the mother was killed at about noon by cervical dislocation. An abdominal incision was made, the uterine cornua were exposed, excised, and placed on a foil surface cooled with ice. Each embryo or fetus was removed individually from surrounding chorionic and amniotic structures and the heart was excised by microdissection under magnification. Both heart and the remaining body were then frozen at dry ice temperature and freeze dried. Weights of freeze-dried hearts were considered to be subject to less error than wet weights of such small structures. Embryos or fetuses were obtained at successive stages of gestation from 10.5 days to birth; thereafter, individuals from birth-dated litters were sampled on Postpartum Days 0.5 through 8.5.

Method of analysis of taurine. Separation and quantitation of taurine were carried out on a Beckman–Altex model 110A high performance liquid chromatography (HPLC) apparatus containing an Ultrasil-Octyl column (250 × 4.6 mm i.d.) of 10-μm particle size (Beckman–Altex), connected to a Gilson Spectra/Glo Fluorometer with a 45-μl flow cell and standard excitation filter of 390 nm and emission filter of 475 nm.

Taurine was quantitated using the technique of Larsen *et al.* (31) with slight modifications to accommodate larger amounts of tissue. Taurine was first extracted from the smaller embryo, fetus, or heart by homogenization in ground glass homogenizers in 300 μl of double-distilled H_2O (DD H_2O). The larger bodies were homogenized with a Polytron homogenizer in 8 ml of DD H_2O with an additional 2 ml used to rinse the homogenate from the Polytron blades. In either case the homogenate was combined with an equal volume of 2% picric acid solution. Large samples were brought to 25 ml with

DD H_2O. All samples were then mixed on a Vortex mixer and allowed to stand for 5 min at room temperature.

Removal of free amino acids other than taurine was accomplished on ion exchange resin columns as described by Larsen *et al.* (31). The total samples from the smaller embryos and hearts were pipeted onto resin in the columns and eluted with three 200-μl rinses of the homogenizing vessels with DD H_2O followed by 1 ml of DD H_2O. The columns were then allowed to run dry. This procedure eluted all of the taurine from the samples. The eluates from very early embryos and hearts were concentrated by lyophilizing and redissolving in 500 μl of filtered DD H_2O. This solution was then used to prepare the fluorescent derivative of taurine immediately prior to analysis.

With larger embryos and hearts, the homogenate–picrate mixture was centrifuged and a 1-ml aliquot of the supernate was applied to the column and eluted with 2 ml DD H_2O and the column was allowed to run dry. The total eluate was collected in a 3-ml volumetric flask and brought to volume just prior to derivatization of an aliquot with *o*-phthalaldehyde (OPA).

The fluorescent adduct was prepared at room temperature by combining equal volumes of either taurine standards or tissue extracts and OPA reagent, prepared as described by Larsen *et al.* (31). The derivatization reaction was allowed to proceed for exactly 1 min at which time a 20-μl aliquot was injected onto the HPLC column.

Taurine was eluted isocratically at 2.0 ml/min using 43% 0.05 *M* NaH_2PO_4, adjusted to pH 5.7 with NaOH, and 57% 0.05 *M* NaH_2PO_4 in 75% methanol/25% DD H_2O. All solvents were passed through a filter of 0.45-μ pore size before use.

Fluorescent peaks and their retention times were visualized on a Beckman strip chart recorder. Quantitation of unknowns was accomplished by comparing peak height measurements against peak heights of three or more concentrations of taurine standards prepared and quantitated daily.

Statistics. Data were analyzed using two-way analysis of variance. P values < 0.01 are considered to be significant. Mean values shown were obtained from measurements on at least three individuals and most were on four or more. Linear trends were analyzed by linear regression and slopes were considered significantly different if one slope was excluded from the confidence level of the other ($P < 0.05$).

Results. Freeze-dried tissues, from animals of known ages, were weighed on a microtorsion balance. The values are shown in the semilogarithmic plot of Fig. 1, which reveals smooth growth curves for both hearts and whole bodies.

Figure 2 shows the log of the total amount of taurine in the body (excluding the heart) and in the heart from Embryonic Day 10.5 through early postpartum development and maturation. These curves are similar to those in Fig. 1, except that cardiac taurine content appears to experience a marked increase at the time of birth.

Figure 3 allows comparison, at known ages, of the concentration of taurine in the whole body (excluding the heart) and concentration of taurine in the heart. Heart and body concentrations of taurine are similar until Day 14.5 of gestation. Body levels of taurine decrease slightly throughout the rest of the period examined. At Day 14.5 of gestation, heart levels of taurine rise slightly and remain relatively constant until birth, whereupon a twofold increase in concentration occurs followed by a decline on Days 2.5–4.5.

Discussion. The coherence of the plot of gestational age vs the log of freeze-dried body weight (Fig. 1) supports the validity of the determinations of the time of fertilization from vaginal plug information assigned to each of the litters. It is perhaps not surprising that the slopes of the pre- and postpartum portions of the curve of body weights differ slightly. Such a difference does not appear to be as marked with heart weights where postpartum values appear to form more of a continuum with the values obtained during gestation.

The semilogarithmic plots of total taurine in the bodies and hearts with time in Fig. 2 in general resemble the weight curves of Fig. 1, with the exception of the marked increase in the total amount of taurine in the heart

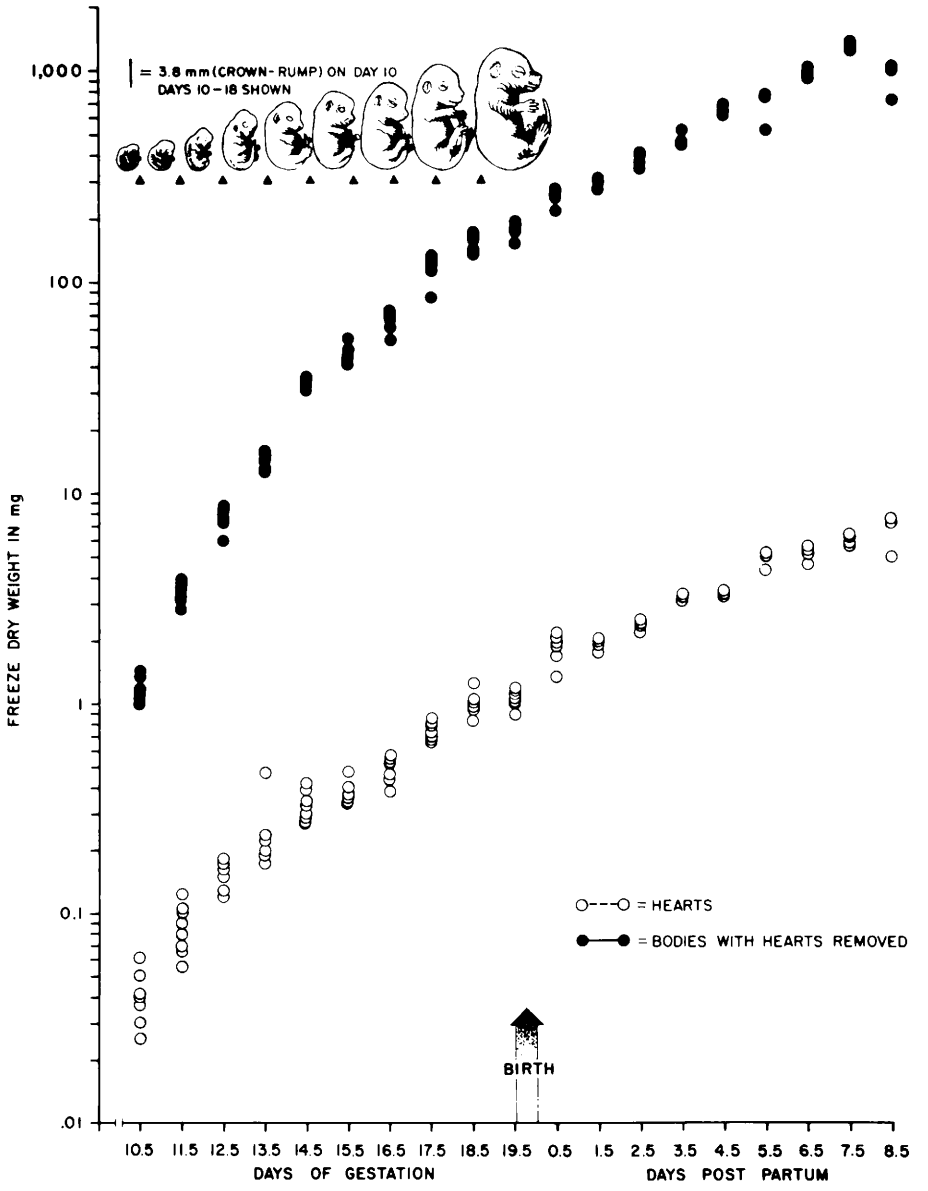

FIG. 1. Logarithm of freeze-dried weights of individual mouse hearts and bodies (with hearts removed) during gestation and early postnatal period. External form of embryos and fetuses from Day 10 through Day 18 are shown in upper corner.

which occurs at birth and continues postpartum. The increase in total body taurine in these mice in the first 8 days postpartum is of the same order of magnitude as that reported by Huxtable (32) for rat pups in the first 3 weeks postpartum. Figure 3 depicts taurine concentration as a function of gestational and postpartum age. The range of taurine concentrations (47–57 nmole/mg freeze-dried wt) for Days 16–19 of gestation compares with the value reported by Grosso *et al.* (26) of 14.1 ± 0.5 nmole/mg fresh tissue which, on a dry weight basis, is 65.6 nmole/mg. A significant ($P < 0.001$) increase in taurine concentration occurs in the heart at birth. This event with its considerable

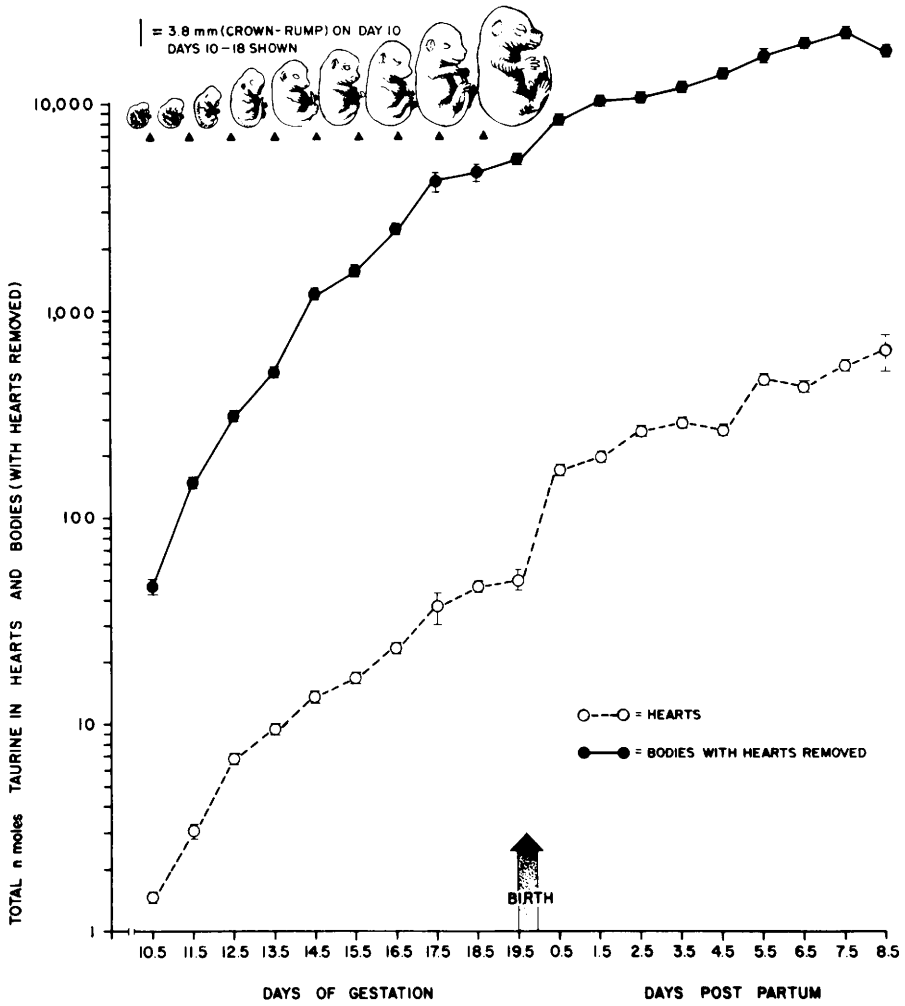

FIG. 2. Logarithm of total taurine content of mouse hearts and bodies (with hearts removed) during gestation and early postnatal period. Values represent means (N = 3–6) with vertical bars indicating ±SEM. External form of the embryos and fetuses from Day 10 through Day 18 is shown in upper corner.

physiological changes coincides with the dramatic increase in taurine concentration measured in hearts from individuals just 6 hr postpartum. This finding contrasts with the report of Gonzales and Awapara (27) who showed a steady increase in taurine concentration throughout incubation and hatching in the chick embryo. However, the 3- to 4-day intervals between their observations could have obscured any sharp, transient increase in taurine concentration. It is also possible that differences between mammalian and avian physiology and nutrition explain these contrasting observations.

The marked increase in taurine concentration in mouse hearts at the time of birth is not attributed to synthesis from cysteine in neonatal tissues, as Yamaguchi (33) has shown that in neonatal rat liver, the activity of cysteine dioxygenase, the enzyme catalyzing the first step in this biosynthesis, first becomes detectable on the eighth postnatal day. Taurine is available to the fetus from the maternal plasma and to the neonate from ingested milk, in which it is present in high concentrations (34). It is possible that the marked increase in cardiac taurine concentration which occurs during the first postpar-

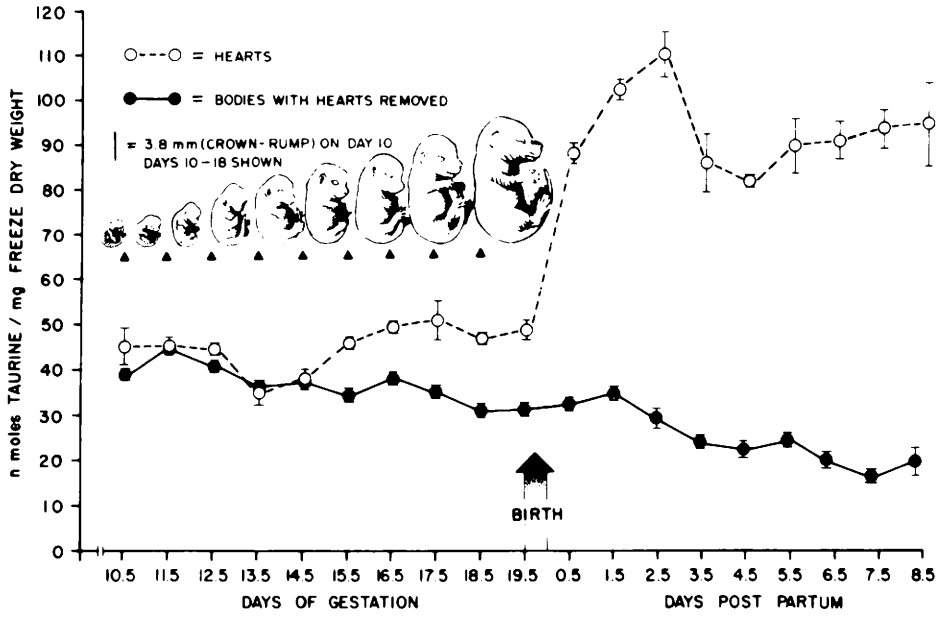

FIG. 3. The concentration of taurine in mouse hearts and bodies (with hearts removed) during gestation and early postnatal period. Values represent means (N = 3–6) with vertical bars indicating ±SEM. External form of embryos and fetuses from Day 10 through Day 18 is shown in upper corner.

tum day reflects taurine intake from nursing; however, it has not been possible to alter the concentration of taurine in the hearts of young adult rats by dietary manipulation (35, 36).

The cardiopulmonary changes occurring at parturition could contribute to the dramatic increase in taurine in cardiac tissue. In the perinatal human, there is a decrease in pulmonary vascular resistance with the first breath due to the effects of increased arterial PO_2 and release of bradykinin, a vasodilator. This is offset by the high pulmonary arterial pressure created by closure of the ductus arteriosus after birth. The major decline in this increased pulmonary arterial pressure is not accomplished until some 2–3 days after birth (37). This causes the right heart to work hard just after birth. If a similar situation occurs in the mouse the rapid increase in cardiac taurine concentration at birth may accompany an increased work load imposed on the neonatal heart. Immediately postpartum the β-adrenergic system is functioning and may be responding to increased work stress by stimulating active transport of taurine into cardiac cells. Huxtable and Bressler (16) have shown that increases in the concentration of taurine in rat hearts accompany increased work loads incident to spontaneous or stress-induced hypertension.

There appears to be a drop in taurine concentrations in mouse hearts several days after birth (Fig. 3). A similar trend has been reported for taurine concentrations in the hearts of postnatal rats (38); concentrations of taurine in hearts were high when first measured 1–2 days postpartum, dropped rapidly, and began a slow rise from Day 5 until adult levels were reached. Our profile hints at a slow rise in cardiac taurine levels beginning at postnatal Day 4. The maximum value for taurine concentration we have measured in the hearts of adult male CF_1 mice is 160 nmol/mg dry wt. This compares with 39.4 nmol/mg fresh tissue (20) which, on a dry weight basis, is about 180 nmol/mg.

If a line is fitted to the data for body taurine in Fig. 3 for the period following Day 14.5 of gestation, it is apparent that taurine concentration is decreasing with time. This could be due to differential growth of tissues that are low in taurine, as suggested

by Gonzales and Awapara (27), or it could be that heart tissue is actively sequestering taurine at the expense of the rest of the body. Huxtable has reported a decrease in the percentage of body taurine residing in the carcass of rat pups from the time of birth to weaning (32). The two curves in Fig. 3 overlap at early time points, which suggests that the taurine concentrations of the heart and of the rest of the body are in equilibrium until Day 14.5 of gestation, after which taurine concentration in the heart is maintained, and increases gradually, possibly by a basal rate of influx as proposed by Huxtable and Chubb (25), while the concentration in the rest of the body decreases. However, the heart may be drawing upon other stores to maintain its concentration of taurine. In humans, the concentration of taurine in fetal plasma appears to decrease steadily during gestation (39) and then rapidly after birth (40). It should be pointed out that this rapid decline in plasma taurine after birth is occurring even though the mother's milk has a high concentration of taurine, which is at a maximum during the first days of lactation (2). This may reflect an increased uptake of taurine by the heart during the first few days of life.

It is interesting to note that Day 15.5 of gestation is close to the time when the embryo becomes responsive to catecholamines. According to Wildenthal (41), the mouse embryo responds to catecholamines throughout the last third of gestation, starting about Day 14. By this time formation of the heart is essentially complete and by Day 15 the spinal cord closely resembles that of the adult. The spinal root ganglia are well developed and the ventral and dorsal roots are properly associated with the cord (42).

Chen *et al.* (30) have demonstrated a density of β-adrenergic receptors, as a percentage of the density in adult mouse hearts, of 65 and 73% for Days 19 and 21 of gestation, respectively, and of 93, 168, and 173% for postnatal Days 1, 3, and 14, respectively. Thus although there is yet no understanding of the reason for the doubling of the concentration of cardiac taurine at birth, it appears that β-adrenergic stimulation in response to a marked increase in work load may contribute to increased taurine uptake by the heart at this time.

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