A mutation in the *Arsb* gene; a mouse model that resembles Mucopolysaccharidosis Type VI (MPS VI)

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Mutation (allele) symbol: $Arsb^{m1J}$

Mutation (allele) name: mutation 1 Jackson

Strain of origin: C57BL/6J

Current strain name: C57BL/6J-$Arsb^{m1J}$/GrsrJ.

Stock #005598 (jaxmice.jax.org)

Phenotype categories: craniofacial, skeletal, neurological, hearing

Abstract
We report here a new mutation in the arylsulfatase B gene (*Arsb*) that causes affected mice to have a phenotype that resembles Maroteaux-Lamy syndrome. ARSB deficiency is better known as the lysosomal storage disorder called Mucopolysaccharidosis Type VI (MPS VI).

Eight-month-old female littermates are shown in this photo. Note the short nose and thick tail of the *m1J*/*m1J* female on the right compared to the +/*m1J* control on the left.

Origin and Description
Our *Arsb* mutation 1 Jackson arose in a C57BL/6J colony that was three generations descended from a male mouse that had been mutagenized with N-ethyl-N-nitrosourea (ENU) from the Neuroscience Mutagenesis Facility at the Jackson Laboratory.

The *m1J* allele is recessive. These mutants have a combination of delayed muscle and nerve degeneration that affects their gait as they age. Mutants can still walk but appear less stable than controls. Mutants also have a skeletal phenotype consisting of a shortened snout, wide-set eyes, a thicker tail and digits and shortened limbs that become more noticeable with age.

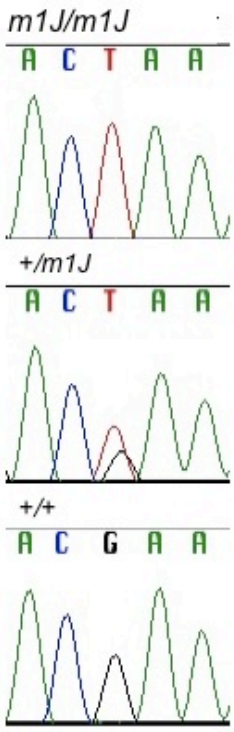
Both male and female homozygotes are fertile. The colony is maintained by brother/sister matings as an *m1J/m1J* X *+/m1J* cross and the reciprocal. Mutants can be poor breeders with small litters, so *+/m1J* X *+/m1J* matings are done when necessary.

A seven-month-old affected *m1J*/*m1J* male is shown on the left of the photo and a sibling +/*m1J* is shown on the right. Note the short nose and the stance of the mutant.

Genetic Analysis

An *m1J/m1J* male was crossed to a BALB/cByJ and obligate heterozygotes were then intercrossed to make F2 mutants that were utilized for linkage analysis. Spleen and tail tips from the affected F2 mutants were collected and stored at -80° C. Using our standard mapping protocol[1], the mutation was mapped to Chromosome 13. There was no recombination with *D13Mit147* at 98.3 Mb (Ensembl release 54). Based on phenotype and map position similarities, *Arsb* located at 94.5 Mb (Ensembl release 54) was thought to be a good candidate gene.

Using the Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm) design program, primers were designed to amplify the first four exons of the *Arsb* gene and were sequenced at The Jackson Laboratory core sequencing facility. A single base change from G to T was detected in exon 2 in the DNA sequence of three mutant mice. This base change modifies the amino acid Glutamic acid as the codon GAA is changed to TAA causing a premature stop codon.



A single base change from G to T was detected *m1J/m1J* mice in exon 2 of the *Arsb* gene. This modifies the codon from GAA (Glutamic acid) to TAA that causes a premature stop codon. The genetic lesion is located at 94,560,057 on chromosome 13.

Biological Characterization

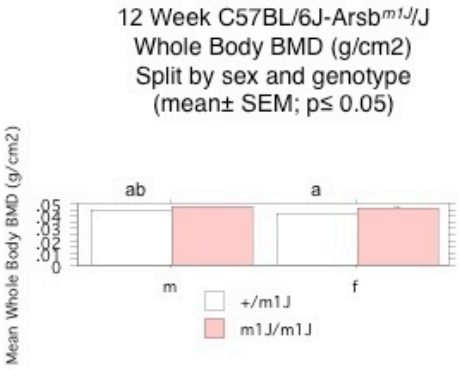
- DEXA analysis of whole body aBMD and body composition of twelve-week-old mice:

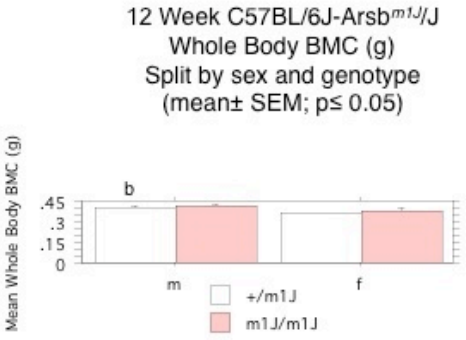
Three-month-old male and female mutant and control mice were collected and whole body, areal bone mineral density (aBMD), bone mineral content (BMC) and body composition (lean, fat and % fat mass) were assessed by PIXImus densitometry (GE LUNAR, Madison, WI; see protocol on the this website). Mutants were significantly smaller overall and had less body fat with more lean than controls; mutants also had significantly greater whole body BMD compared to heterozygotes.

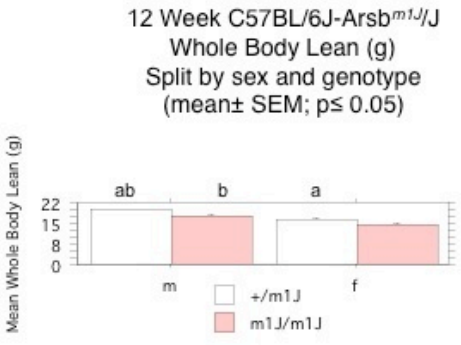
Table 2: PIXImus Densitometric Measurements of Twelve-week-old C57BL/6J-Arsb^{m1J}/J
(mean ± SEM; ^{ab}p≤ 0.05)

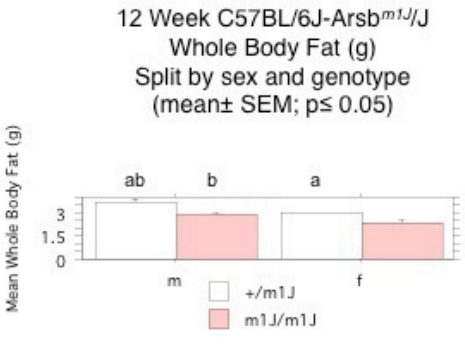
Measurements	Male *+/m1J* (n=5)	Male *m1J/m1J* (n=6)	Female *+/m1J* (n=4)	Female *m1J/m1J* (n=6)
Whole Body BMD (g/cm2)	.0441± .00074^{ab}	.0468 ± .00064	.0416 ± .00031^{a}	.0456 ± .00124
Whole Body BMC (g)	.402 ± .0103^{b}	.414 ± .0166	.365 ± .0037	.378 ± .0165
Whole Body Lean (g)	19.9 ± .34^{ab}	17.8 ± .55^{b}	16.2 ± .37^{a}	14.4 ± .46
Whole Body Fat (g)	3.6 ± .299^{ab}	2.9 ± 1.67b	3.0 ± .05^{a}	2.4 ± .12
Total Mass (g)	23.5 ± .58^{ab}	20.7 ± .66^{b}	19.2 ± .33^{a}	16.8 ± .55
% Fat	15	14	16	14
Skull BMD (g/cm2)	.1122 ± .00113^{a}	.1274 ± .00296	.1141 ± .00106^{a}	.1252 ± .00298
Skull BMC (g)	.260 ± .0046^{a}	.309 ± .0159	.256 ± .0046	.291 ± .0120
Skull BMD/ Body BMD	2.5461 ± .06222^{ab}	2.7212 ± .04324	2.7400 ± .01648	2.7446 ± .03159

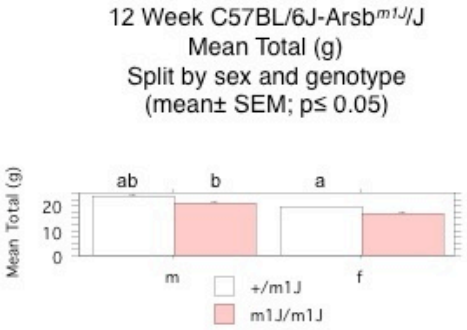
^{a} p≤ 0.05 *+/m1J* vs. *m1J/m1J* within sex
^{b} p≤ 0.05 female vs. male within genotype

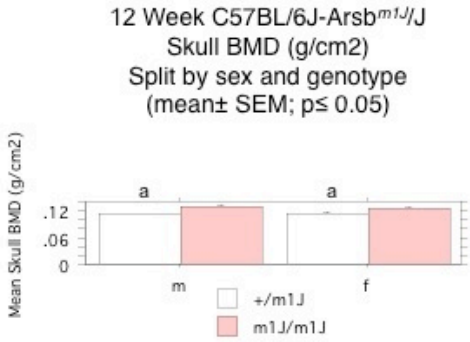


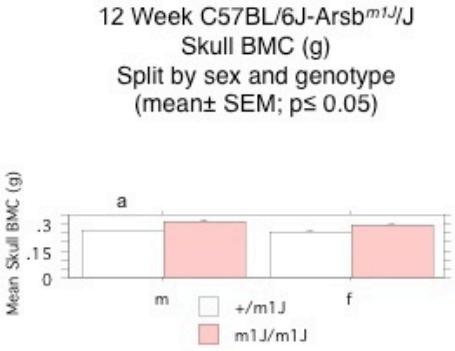


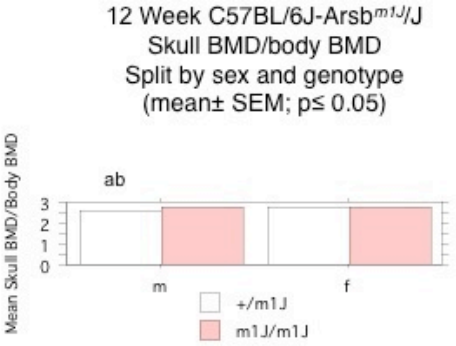












- Craniofacial morphology of twelve-week-old mice:
Three-month-old male and female mutant and control skulls were collected and were prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). Morphological measurements of the skull (See protocol[1]) were made using digital calipers (Stoelting, Wood Dale, Ill) with previously established landmarks (Richtsmeier, 2000). Skull BMD was significantly greater in male and female mutants and skull BMC was significantly greater in male mutants. Mutants from both sexes had significantly shorter noses and upper jaw lengths (see data below).

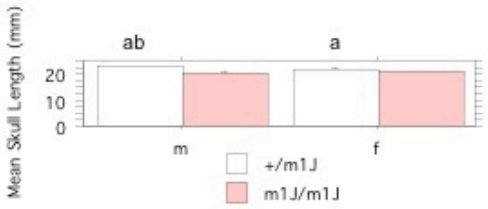
Table 1: Digital Caliper Measurements in Millimeters and Calculated Ratios of Twelve-week-old C57BL/6J-*Arsb*^{m1J}/J Skulls Stained with Alizarin Red (mean ± SEM; $^{ab}p \leq 0.05$)

Measurements	Male *+/m1J* (n=5)	Male *m1J/m1J* (n=6)	Female *+/m1J* (n=4)	Female *m1J/m1J* (n=6)
Skull Length	22.59 ± .191^{ab}	20.32 ± .237	21.83 ± .225^{a}	20.63 ± .261
Nose Length	15.78 ± .270^{a}	13.43 ± .368	15.21 ± .253^{a}	14.09 ± .296
Skull Height	10.94 ± .167	10.69 ± .182	10.50 ± .078	10.58 ± .072
Skull Width	10.75 ± .219	11.00 ± .222	10.47 ± .172	10.76 ± .062
Inner Canthal Distance	6.60 ± .106	6.34 ± .148	6.36 ± 0.076	6.22 ± .130
Lower Jaw Length	10.96 ± .056	10.68 ± .147	10.74 ± 0.143	10.61 ± .044
Upper Jaw Length	16.07 ± .164^{a}	13.45 ± .711	15.57 ± 0.330	14.48 ± .343
Jaw Length Ratio	1.47 ± .011^{a}	1.26 ± .056	1.45 ± 0.024	1.37 ± .030
Skull/Nose Length Ratio	1.43 ± .017^{a}	1.52 ± .028	1.44 ± .023	1.47 ± .019
Skull Height/Length Ratio	.49 ± .010^{a}	.53 ± .009	.48 ± .005a	.51 ± .009
Skull Length/Width Ratio	2.10 ± .039^{a}	1.85 ± .040	2.09 ± .052a	1.92 ± .022
Skull Height/Width Ratio	1.02 ± .016^{a}	.97 ± .008	1.00 ± .016	.98 ± .008
Right Ear Pinna Length	13.36 ± .133	13.31 ± .224*	13.55 ± .166	13.43 ± .204

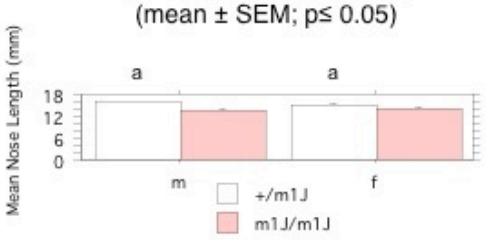
*n=5

^{a} $p \leq 0.05$ *+/m1J* vs. *m1J/m1J* within sex
^{b} $p \leq 0.05$ female vs. male within genotype

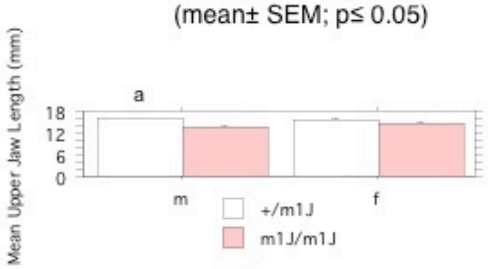
12 Week C57BL/6J-Arsb^{m1J}/J
Skull Length (mm)
Split by sex and genotype
(mean± SEM; p≤ 0.05)



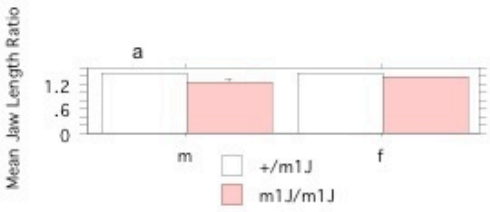
12 Week C57BL/6J-Arsb^{m1J}/J
Nose Length (mm)
Split by sex and genotype
(mean ± SEM; p≤ 0.05)



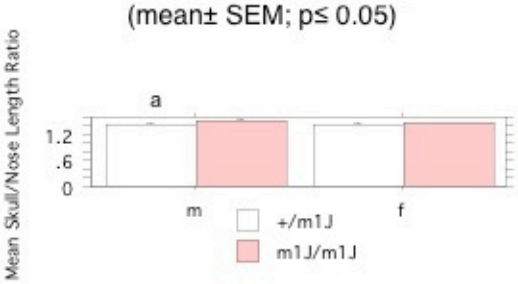
12 Week C57BL/6J-Arsb^{m1J}/J
Upper Jaw Length (mm)
Split by sex and genotype
(mean± SEM; p≤ 0.05)



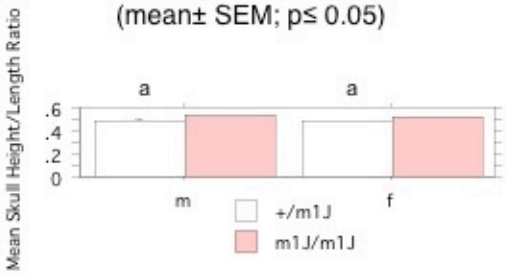
12 Week C57BL/6J-Arsb^{m1J}/J
Mean Jaw Length Ratio
Split by sex and genotype
(mean± SEM; p≤ 0.05)



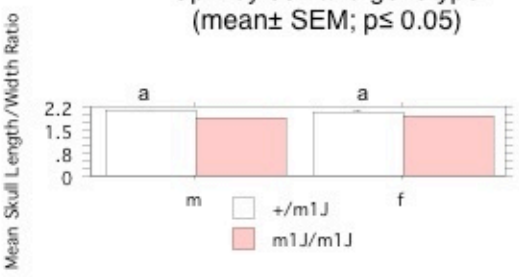
12 Week C57BL/6J-Arsb^{m1J}/J
Skull/Nose Length Ratio
Split by sex and genotype
(mean± SEM; p≤ 0.05)



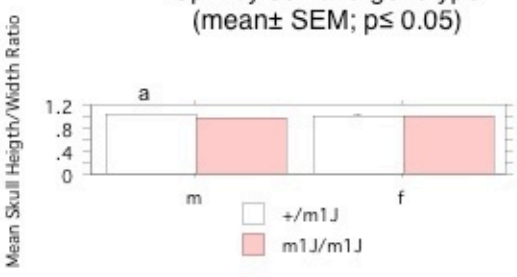
12 Week C57BL/6J-Arsb^{m1J}/J
Skull Height/Length Ratio
Split by sex and genotype
(mean± SEM; p≤ 0.05)



12 Week C57BL/6J-Arsb^{m1J}/J
Skull Length/Width Ratio
Split by sex and genotype
(mean± SEM; p≤ 0.05)



12 Week C57BL/6J-Arsb^{m1J}/J
Skull Height/Width Ratio
Split by sex and genotype
(mean± SEM; p≤ 0.05)



Pathology

A routine pathology screen[2] on a two-and-a-half month old mutant mouse showed clumped irregular cartilage in the growth plate of the knee and the spiral ganglion. An eight-month-old mutant revealed focal muscle degeneration and degeneration of peripheral nerves with macrophage infiltration. A 14-month-old male mutant had peripheral nerve degeneration along with spinal arthritis and strange looking vacuoles in the connective tissue around the spine; this mouse also had otitis media and a histologically normal heart but the aorta appeared larger than normal.

To confirm lysosomal storage, liver, kidney, lung and rear legs were stained with Mowry's colloidal iron stain. In a 26-week-old mutant, there were Kupffer cells in the liver that the littermate control did not show and there were some macrophages. In a 47-week-old mutant, Kupffer cells were in the bone marrow and the macrophages in the liver were larger (see slides 1, 2, and 3).

The auditory-evoked brainstem response[3] (ABR) was used to assess hearing in seven *m1J/m1J* and three *m1J*/+ all 40 days of age or older. All mutants had severe hearing loss while their littermate controls had good hearing. The mucopolysaccaridoses are known to have associated hearing deficits, as do *Arsb*^{m1J} mutant mice. A clinical eye exam (http://eyemutant.jax.org/screening.html) of two *m1J/m1J* and two *m1J*/+ mice at three-and-a-half months old revealed no abnormalities except for one male *m1J/m1J* that had white in the cornea of one eye determined to be a developmental condition of C57BL/6 and not the result of the mutation.

Discussion

Maroteaux-Lamy syndrome in humans is characterized by short stature, stiff joints, dysmorphic facial features and cardiac abnormalities due to disruption in the arylsulfatase B gene. The C57BL/6J-*Arsb*^{m1J}/GrsrJ mouse exhibits these symptoms phenotypically and the same gene responsible for Maroteaux-Lamy syndrome is disrupted in this mouse model.

Acknowledgements

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References

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Protocols

[1]Standard Protocols and Procedures of The Jackson Laboratory Craniofacial Mutant Resource: Mouse Colony Maintenance

Craniofacial Resource mice are housed in 51 square inch polycarbonate boxes, on bedding composed of sterilized shavings of Northern White Pine, under 14:10 hour light:dark cycles. A diet of autoclaved NIH 31 (6% fat diet, Ca:P of 1.15:0.85, 19% protein, vitamin and mineral fortified; Purina Mills International, Richmond, IN) and water acidified with HCl to achieve a pH of 2.8-3.2 (which prevents bacterial growth)

are freely available. Mouse colony maintenance and use is reviewed and approved by The Jackson Laboratory Institutional Animal Care and Use Committee and is in accordance with The National Institutes of Health guidelines for the care and use of animals in research.

PIXImus Densitometry

PIXImus scans (PIXImus, LUNAR, Madison, WI) which provide skeletal and body composition data such as areal bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g), body mass (g), lean mass (g), fat mass (g), and percent fat mass, are completed on groups of 6 male and 6 female 12-week-old mutant and control mice. The skulls and bodies are scanned separately to provide independent data on skull aBMD and BMC and body BMD and BMC. The PIXImus small animal densitometer (DEXA) has a resolution of 0.18 x 0.18 mm pixels and is equipped with software version 1.46 . The PIXImus is calibrated routinely with a phantom utilizing known values, and a quality assurance test is performed daily. The variability in precision for measuring total body BMD is, less than 1%, and approximately 1.5% for specialized regions such as the skull. The correlation between PIXImus BMD measurements of 614 lumbar vertebrae compared to peripheral quantitative computerized tomography (pQCT) measurements was found to be significant ($p<0.001$; $r=.704$) (Donahue, 1999).

Faxitron X-rays

X-rays at 5X magnification of the skull and at 3X magnification of the body of a male and female mutant and control at 12 weeks of age are obtained using a Faxitron MX20 cabinet X-ray (Faxitron X-Ray Corp., Wheeling, IL. USA) and Kodak Min-R 2000 mammography film (Eastman Kodak Co., Windsor, CO, USA). X-rays are then analyzed to determine the specificity of the skeletal phenotype.

Skull Preparation

Skulls of 6 male and 6 female mutants and controls are collected at 12 weeks of age, prepared by incomplete maceration in potassium hydroxide, stained with alizarin red, and stored in undiluted glycerin (Green, 1952). During the collection process, right ear pinnae are measured with digital hand calipers (Stoelting, Wood Dale, IL, USA).

Hand Caliper Skull Measurements

Seven measurements taken with hand held digital calipers are used routinely to define skull morphology at The Jackson Laboratory's craniofacial resource. These measures have a high degree of accuracy and precision in our hands and are able to discriminate differences between mutant and control skull characteristics. Our linear measures have been added to those illustrated by Dr. Joan Richtsmeier in her paper characterizing craniofacial differences in mouse models of Down Syndrome using three dimensional anatomical landmarks (Richtsmeier, 2000. Dev. Dyn. Feb; 217(2):137-45). Skulls are cleared with potassium hydroxide and stained with alizarin red dye in preparation for caliper measurements to be taken.

Skeletal Preps

In many cases whole skeletons of mutant and control mice are cleared in 1% KOH, stained with alizarin red, stored in glycerin (Green, 1952) and then evaluated for skeletal malformations. Malformations found can indicate that the craniofacial phenotype is part of a greater syndrome.

Data Analysis

Hand caliper skull measurements and PIXImus skeletal and body composition data are evaluated using StatView 4.5 software (Abaccus Cary, NC) for Macintosh computers. Differences are considered significant when $p < 0.05$.

Molecular Mapping

Molecular mapping is completed using DNA extracted from tail or spleen through a Hot Sodium and Tris (HotSHOT) protocol (Truett, 2000). Primer pairs (MapPairs, Research Genetics, Huntsville Ala.) of microsatellite markers are used to establish and refine the initial mapping location. PCR products are visualized and scored via gel electrophoresis and ethidium bromide staining. Linkage analysis and recombination frequencies are calculated via the Map Manager computer program (Manly, 2001) and compared to known and predicted gene location data and marker location data published in the Celera and Ensembl genome databases and the Mouse Genome Informatics website.

Sequencing

Primers are designed using published cDNA sequences from the Ensemble or Celera mouse genome databases. DNA is amplified through PCR and separated via gel electrophoresis. The resultant products are then removed and purified with QIAquick Gel Extraction Kits (Qiagen, Inc., Valencia, CA, USA) and sequenced using an Applied Biosystems 373A DNA Sequencer and an optimized DyeDeoxy Terminator Cycle Sequencing method.

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[2] Standard Histology Protocol used in the Mouse Mutant Resource

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.

[3] Auditory-Evoked Brainstem Response (ABR) Thresholds

ABR thresholds in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.