Differences in cerebral morphology in 2 stocks of mutant mice heterozygous for the loop-tail (*Lp*)-gene[1]

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Summary. Adult loop-tail heterozygotes (*Lp*/+) from a stock of *Lp*-mice which consistently fail to show head wobbling exhibit normal brain morphology with respect to size and shape of lateral ventricles and nearby nuclei. Loop-tail heterozygotes from a head wobbling stock of *Lp*-mice show enlargement and deformity of the lateral ventricles.

The loop-tail (*Lp*) mutant mouse has been the subject of several studies concerned with morphological manifestations of the gene in the heterozygous and homozygous conditions[2-8]. Loop-tail homozygotes (*Lp*/*Lp*) show an extensive failure of the neural tube to close as well as axial skeletal abnormalities. Since homozygotes die at birth, studies on behavioral manifestations of the gene have been confined to the heterozygous condition (*Lp*/+)[9,10]. Of interest has been the finding that loop-tail heterozygotes which display a wobbling of the head also show abnormalities in the lateral ventricles and nearby nuclei of the cerebral hemispheres[11].

In our colony, the *Lp*-gene is on 2 different genetic backgrounds. One genetic stock has been derived from mutant *Lp* animals obtained from the Jackson Laboratory (hereafter termed 'agouti'), and the *Lp*-heterozygotes of this group show the characteristic head wobble. The other stock consists of descendants from a loop-tail stock of albino mice obtained originally from Dr Kathryn Stein at Mount Holyoke College; in our colony, these albino *Lp*-mutants do not show a distinctive head wobble. In view of the possible association between head wobble and cerebral pathology[11], loop-tail heterozygotes from both mutant stocks in our colony were studied grossly and microscopically for evidence of cerebral defects, particularly in the lateral ventricles and nearby nuclei.

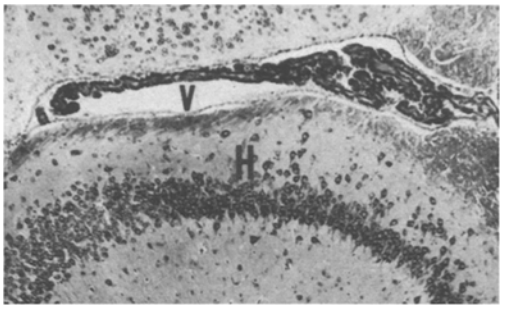

Fig. 1. Portion of horizontal brain section of *Lp*-heterozygote (*Lp*/+) from nonwobbling stock. Note narrow ventricle, *V*. *H* hippocampus. ×52.

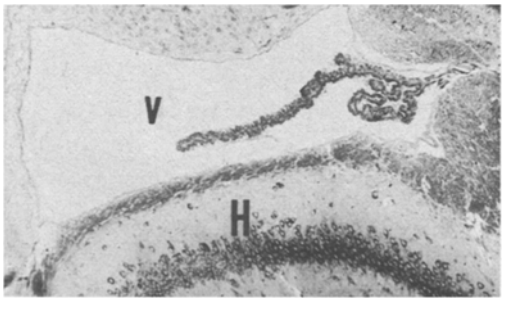

Fig. 2. Portion of horizontal brain section of *Lp*-heterozygote (*Lp*/+) from wobbling stock. Note enlarged ventricle, *V*. *H* Hippocampus. ×50.

Materials and methods. Female and male adult normal (+/+) and loop-tail heterozygotes (*Lp*/+), of the head wobbling agouti line and nonwobbling albino line were selected for histological studies on the brain. The animals ranged in age from 11 weeks to 24 weeks. Each whole brain was removed, fixed in formalin, embedded in paraplast, and sectioned at 10 μm in the horizontal, transverse or sagittal plane. Particular care was taken in orienting each brain so as to avoid oblique sections. The sections were stained with hematoxylin and eosin or cresyl violet-luxol fast blue. The table summarizes the numbers and types of brains used in this study.

Results. In the nonwobbling albino stock of loop-tail mice, no gross differences could be seen in the size or external morphology of the brain from females or males of heterozygous loop-tail (*Lp*/+) and homozygous normal (+/+) individuals. Neither could any obvious distortions in the size and shape of the ventricles be detected in comparisons of microscopic sections of the *Lp*/+- and +/+-brains. The lateral ventricles of the *Lp*/+-brains showed the narrow slit-like appearance typical of normal brains (figure 1). The adjacent nuclei, septal area and hippocampus also appeared to be normal.

In the head wobbling agouti stock of loop-tail mice, the brains of the heterozygotes (*Lp*/+) likewise showed no gross differences in size or external morphology from those of the homozygous normal (+/+) individuals. However, the microscopic sections revealed that in the cerebrum the lateral ventricles of the *Lp*/+-brains were enlarged and distorted (figure 2). In most cases, these defects occurred bilaterally, although in one *Lp*/+-brain only one lateral ventricle was affected. In some cases, the third ventricle was also slightly enlarged. Distortions were likewise evident in the overall shape of the septal area and hippocampus in the *Lp*/+-brains of the head wobbling stock, presumably due to the encroachments of the enlarged ventricles. Histological differentiation and

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stratification appeared to be normal in these regions.

Discussion. Although the behavioral trait of head wobbling has generally been included in the syndrome of anomalies attributable to the *Lp*-gene[12,13], early studies on this mutant noted that the expression of wobbling was somewhat variable, in that it occurred not only in the phenotypic heterozygotes but even in some straight-tailed individuals which later produced loop-tailed offspring[2]. This variability suggested incomplete penetrance of the *Lp*-gene in the heterozygous condition or the effect of modifying genes reducing the expression of the tail defect. However, in our wobbling stock of *Lp*-mutants, the head wobble has not been observed in straight-tailed individuals. Since both our wobbling and nonwobbling *Lp*-stocks consistently show the other cardinal characteristics typical of the *Lp*-defects, i.e., looped tails in the heterozygotes and extensive rachischisis and skeletal anomalies in the homozygotes (*Lp*/*Lp*), this strongly suggests that the expression of the head wobbling trait may be affected by the genetic background on which *Lp* is found. Our results confirm those of Van Abeelen and Raven[11], who were the first to observe that the head wobble appears to be associated with the presence of enlarged ventricles in the cerebral hemispheres, since our nonwobbling stock failed to exhibit comparable cerebral defects. Although the condition of the vestibular apparatus was not examined in our studies, earlier investigations showed that the ear appeared to be normal in *Lp*/+-individuals with head wobble[3]. It is of interest that behavioral studies on head wobbling *Lp*/+-mice have also revealed impairment of various motor skills[9,10]. Further studies comparing locomotor skills of our head wobbling and nonwobbling stocks of *Lp*-mutants would thus seem to be warranted in order to correlate more closely the brain defect with the presence or absence of specific behavioral deficiencies.

Number of brains studied from 2 stocks of loop-tail mutant mice

	Agouti (head wobbler)		Albino (nonwobbler)	
	+/+	*Lp*/+	+/+	*Lp*/+
Female	4	3	3	3
Male	2	3	3	3

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A quantitative analysis of plasma osmotic pressure during metamorphosis of the bullfrog, *Rana catesbeiana*[1]

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Summary. The plasma constituents contributing to osmotic pressure are, in decreasing order: Na^+, Cl^-, HCO_3^-, K^+, glucose, amino acids, urea and protein. Plasma osmotic pressure increases from 180 mmoles/l to 200 mmoles/l throughout development.

Plasma osmotic pressure and its contributing components were quantitated throughout the life span of R. catesbeiana in an attempt to help establish the optimum conditions for bullfrog larval tissue culture. No one has succeeded in establishing longterm bullfrog larvae tissue culture[2], but in successful amphibian tissue cultures it has been recognized that proper osmotic pressure conditions had to be present[3,4].

Materials and methods. Tadpoles were collected from local ponds and adults were bought from commercial suppliers. Animals were maintained in declorinated tapwater at 20 ± 2 °C. Animals were anesthetized in tricane methane sulfonate (MS-222) and blood obtained as previously described[5]. The whole blood was centrifuged to remove blood cells and plasma stored at −20 °C until analysis. The total osmotic pressure was determined by freezing point depression[6]. The individual plasma constituents were measured with an autoanalyzer[7]. Protein determinations were by the Lowry method.

Results. Irrespective of the size or stage[8] of the animal, the hematocrit is around 30% (table 1). Osmotic pressure of the plasma increases gradually from 181 mmoles/l in young stages, to a high of about 200 mmoles/l in late stages and adult (table 1). The difference between these values is significant at the 5% level. This increase can also be indicated by the percent of animals which have an osmotic pressure of 200 mmoles/l or more; none had these high values at stage XIII or lower, 9% between stages XVI and XIX showed this high pressure, and 36% of stage XX or older showed the high values in osmotic pressure.

The major contributor to osmotic pressure in plasma is Na^+, accounting for about 50% of the total pressure (table 2). During larval development the Na^+ levels increase about 10%, and while K^+ also increases, it accounts

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