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

12 H. Gruneberg, The Pathology of Development. A Study of Inherited Skeletal Disorders in Animals, p. 153. Blackwell, Oxford 1963.

13 R. L. Sidman, M. C. Green and S. H. Appel, Catalog of the Neurological Mutants of the Mouse. Harvard University Press, Cambridge 1965.

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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2 M. A. Monnickendam and M. Balls, Experientia *29*, 1 (1973).

3 J. J. Freed and L. Mezger-Freed, in: Cell Physiology, vol. IV, p. 19. Ed. D. Prescott. Academic Press, N.Y. 1970.

4 M. Balls and R. S. Worley, Exp. Cell Res. *76*, 333 (1973).

5 J. J. Just and B. G. Atkinson, J. exp. Zool. *182*, 271 (1972).

6 Osmometer: Advanced Instrument Co., Newton Highland, Mass., USA.

7 Autoanalyzer: Model SMA 6/60, Technicon Instruments Corp., Tarrytown, New York, USA.

8 A. C. Taylor and J. J. Kollros, Anat. Rec. *94*, 7 (1946).

for less than 3% of the osmotic pressure. Cl^- is responsible for about 30% of the osmotic pressure while HCO_3^- accounts for 15% or less.

The organic compounds did not contribute markedly to the total osmotic pressure, but glucose gave the greatest proportion. Although the tail disappears in late metamorphosis (stages XX–XXIV) the amino acid levels do not show a marked increase. Urea and protein concentrations more than double from the early stages of development (less than stage X) to the late stages.

Discussion. These results agree with other investigations which suggested a rise in the osmotic pressure of plasma during amphibian development[11,12]. Although previous investigations did not show a continuous rise in the osmotic pressure, by using large sample sizes our results do show such a rise throughout development (table 1) and individual plasma constituent levels support a continuous rise (table 2).

As is true for most vertebrates[13] the major contributors to osmotic pressure in R. catesbeiana tadpole plasma are the inorganic constituents, especially Na^+ and Cl^- (tables 1 and 2). Although Na^+ and Cl^- concentrations are higher in adult and late larval amphibians when compared to young larvae from the same species, they are not as high as might be expected by the appearance of a Na^+ pump in the skin of amphibians after metamorphic cli-

9 J. J. Just, R. N. Gatz and E. C. Crawford, Jr, Respir. Physiol. *17*, 276 (1973).
10 J. J. Just and G. W. Robinson, Comp. Biochem. Physiol. (accepted 1977).
11 M. S. Gordon and V. A. Tucker, J. exp. Biol. *42*, 437 (1965).
12 R. H. Alvarado and A. Moody, Am. J. Physiol. *218*, 1510 (1970).
13 P. J. Bentley, Endocrines and Osmoregulation, p. 1. Springer-Verlag, New York 1971.

Table 1. Weight, hematocrit and plasma osmotic pressure of *R. catesbeiana*

T–K stages	Weight No.	g[a]	SE[b]	Hematocrit No.	Percent[a]	SE[b]	Osmotic pressure No.	mOsmol[a]	SE[b]
II–VII	75	2.95	0.22	74	29.2	0.7	17	181.0	2.4
X–XIII	25	9.49	0.73	24	28.5	1.1	24	181.4	2.2
XVI–XVII	13	20.41	2.49	13	33.9	1.7	11	185.6	3.0
XVIII–XIX	37	14.97	0.72	22	28.5	1.2	33	187.6	1.8
XX–XXI	28	13.28	0.89	18	28.3	1.2	27	195.0	1.3
XXII–XXIII	45	8.74	0.40	15	35.0	1.7	26	200.0	2.2
XXIV–XXV	14	8.62	0.76	14	28.4	2.6	16	194.2	3.3
Adult	4	221.0	27.0	4	35.3	1.8	4	197.8	7.0

[a] Mean. [b] One SE of the mean.

Table 2. Plasma constituents contributing to the osmotic pressure in *Rana catesbeiana* larvae and adult

A. Inorganic

T–K stages	Na^+ No.	mean[a] ± SE[b]		K^+ No.	mean ± SE		Cl^- No.	mean ± SE		HCO_3^-[c] No.	mean ± SE	
II–VII	18	96.1	2.1	18	3.37	0.09	18	60.3	3.3			
X–XIII	24	98.9	2.0	24	3.15	0.07	24	58.7	2.0			
XVI–XVII	13	103.9	1.8	13	3.37	0.13	13	60.3	2.4			
XVIII–XIX	33	99.4	1.2	33	3.25	0.09	33	56.5	1.5	16[c]	8.4	0.8
XX–XXI	27	103.8	1.3	27	3.33	0.08	27	59.4	1.6	18[c]	14.9	1.2
XXII–XXIII	26	108.5	1.4	26	4.13	0.10	26	58.8	1.4	9[c]	22.1	2.7
XXIV–XXV	17	104.7	2.6	17	4.00	0.21	17	59.5	3.1	4[c]	24.4	3.5
Adult	4	103.0	3.1	4	3.95	0.22	4	38.8	5.0	6[c]	27.6	2.0

B. Organic

	Glucose			Amino acids[d]			Urea			Protein[e]		
II–VII	12	2.45	0.25	3	1.21	0.23	11	0.28	0.08	18	0.18	0.01
X–XIII	20	1.53	0.19	2	1.17	0.09	15	0.33	0.05	24	0.18	0.01
XVI–XVII	13	2.99	0.58	2	1.52	0.10	10	0.38	0.04	13	0.28	0.02
XVIII–XIX	30	2.19	0.26	2	2.27	0.15	25	0.44	0.04	33	0.25	0.01
XX–XXI	25	2.14	0.23	2	2.04	0.28	18	0.58	0.06	27	0.34	0.02
XXII–XXIII	26	2.95	0.22	2	2.00	0.23	22	0.58	0.04	26	0.42	0.02
XXIV–XXV	17	2.41	0.35	2	1.68	0.08	10	0.43	0.09	17	0.40	0.02
Adult	3	0.84	0.14	3	0.98	0.03	3	1.39	0.48	4	0.40	0.06

[a] Means are expressed as mequiv/l. [b] One SE of the mean. [c] See Just et al.[9]; respectively stages XVIII–XX, XXI–XXII, XXIII–XXIV, XXV and adult. [d] See Just and Robinson[10]; to convert mg/l to mequiv/l the average mol.wt (140) was used. [e] To convert mg/l to mequiv/l the percent mol.wt of albumin (68,000) was used.

max[12,14,15]. There is no drastic increase in these ions because the gills, the organs responsible for ion accumulation mechanism in tadpoles, are degenerating simultaneously with the acquisition of the skin transporting mechanisms[9,16,17]. The third most abundant inorganic component is HCO_3^- (table 2). Carbonate is important physiologically because it contributes significantly to the increase in osmotic pressure (tables 1 and 2). An increase in HCO_3^- in the blood occurs as the gills degenerate and the lungs begin to function, resulting in higher levels of CO_2 levels in the blood[9].

Although some organic constituents do increase during metamorphosis, they do not constitute a major proportion of the increase in plasma osmotic pressure, because no more than 6% of the total osmotic pressure originates with organic constituents (table 2). The changes in individual constituents (i.e., glucose, amino acids, urea) all point to the fact that adults were undernourished on arrival from commercial suppliers.

By adding the measured plasma constituents in all stages the percent of the total osmotic pressure (table 1) contributed by these constituents was determined. The measured plasma constituents account for an average 96% of the total plasma osmotic pressure at all time periods studied, ranging from 90% (adult) to 102% (stages XXIV–XXV), indicating no major contributor was overlooked.

By comparing amphibian culture[2] and amphibian plasma osmotic pressure[13], it is evident that except for one[18], successful cultures were achieved with media which was either hypotonic or isotonic to plasma. The information presented here not only permits the choice of proper osmotic pressure for in vitro studies of tadpole tissues, but also identifies the major constituents necessary for the media.

14 E. I. J. Adolph, Exp. Zool. *47*, 179 (1927).
15 R. E. Taylor and S. B. Barker, Science *148*, 1612 (1965).
16 T. H. Dietz and R. H. Alvarado, Am. J. Physiol. *226*, 764 (1974).
17 B. G. Atkinson and J. J. Just, Devl. Biol. *45*, 151 (1975).
18 D. L. Stocum, Devl Biol. *18*, 441 (1968).

Ultrastructural autoradiographic study of blast cells in the mouse thymus. Interest for radioleukemia research

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Summary. Ultrastructural autoradiographic studies of mouse thymic blast cells after H3 Tdr injection show that their fine nuclear structure is related to their position in the cell cycle. The variations in the composition of the subcapsular blast cell population during radiation-induced leukemogenesis indicate kinetic changes in thymic lymphopoiesis, which are probably due to the oncogenic process.

Murine thymic lymphomas induced by irradiation result from the neoplastic transformation of some lymphoid subcapsular blast cells by an oncornavirus[1]. Ultrastructural investigations have revealed the morphological heterogeneity of this population. According to the nuclear structure, 3 cell aspects can be distinguished: lymphoblasts, ring-shaped nucleolus (RSN) cells and X-cells[2] (figure). Their relative proportion varies during the successive phases of atrophy and regeneration which precede the development of lymphoma[2,3]. In other models, it has been shown by using radioautographic and cytochemical methods that the fine structure of the nucleus can be related with the position of the cell in the cycle[4]. In order to control whether a similar explanation can be proposed for the thymic blast population, we have investigated by ultrastructural radioautography the incorporation of H3-thymidine (H3 Tdr) in the normal thymus.

Material and methods. 35-day-old female C57 BL mice are injected with 9 μCi/g of H3-thymidine (spec. act. 5 Ci/mM). Animals are sacrificed 15, 30, 60 and 120 min later. Thymus is fixed for electron microscopy[2]. 1000-Å thick sections of subcapsular zone are put on formvar

1 H. S. Kaplan, Ser. Haemat. *7*, 94 (1974).
2 J. Boniver, M. Delrez, L. J. Simar and J. Haot, Beitr. path. Anat. *150*, 229 (1973).
3 J. Boniver, L. J. Simar, R. Courtoy and E. H. Betz, in preparation.
4 K. Tokuyasu, S. C. Madden and L. J. Zeldis, J. Cell Biol. *39*, 630 (1968).

Labelling index of thymic subcapsular blast cells at various delays after a H3 Tdr injection

		Blast cells	Lymphoblasts	X-cells	Ring-shaped nucleolus cells
Percentage in the blast cell population		100	58.8 ± 5.8	22.0 ± 3.7	19.0 ± 4.3
Labelling index after H3 Tdr	15 min	49.5 ± 7.1	58.35 ± 8.35	77.5 ± 2.5	23.02 ± 4.3
	30 min	78.5 ± 1.14	78.3 ± 1.4	88.7 ± 11	33.3 ± 9.6
	60 min	74.3 ± 3.5	57.1 ± 5.4	90.2 ± 3.8	32.7 ± 3.2
	120 min	73.6 ± 4.98	70.3 ± 1.6	100	60.5 ± 5.5
	8 h	38.0 ± 5.9	43.0 ± 4.1	42.6 ± 7	35.0 ± 3.5