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.

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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

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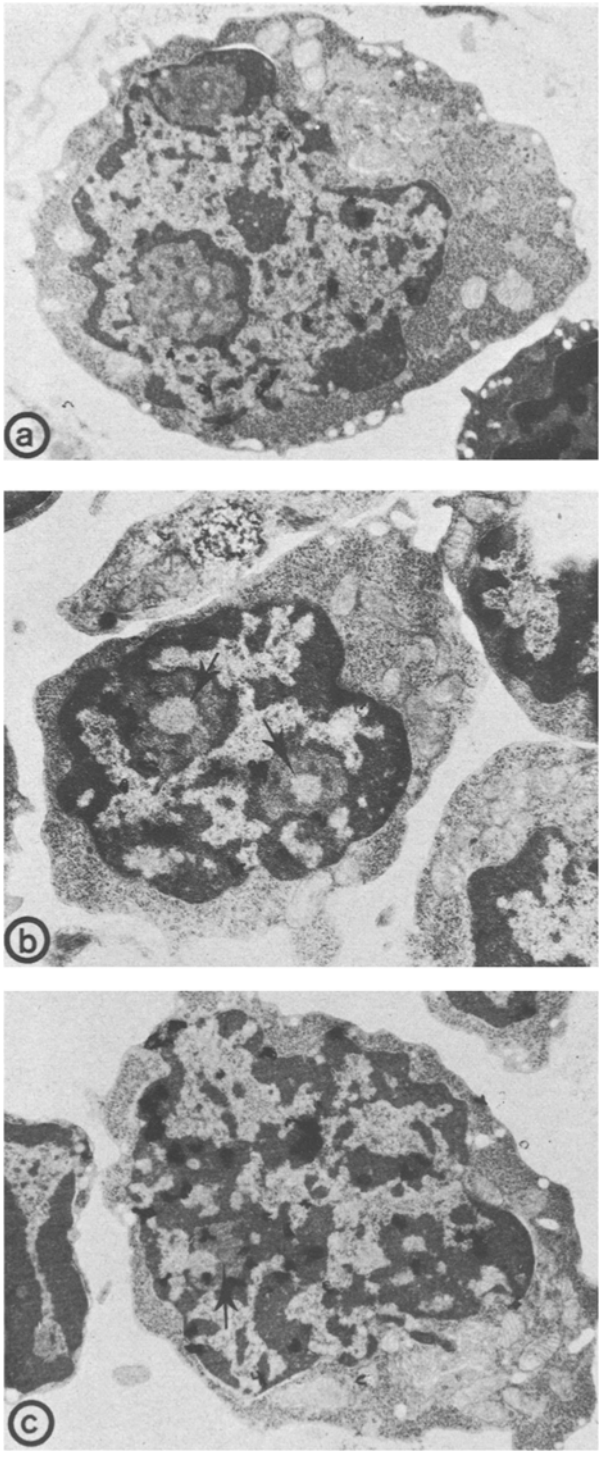
Labelling index of thymic subcapsular blast cells at various delays after a H3 Tdr injection

		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



Ultrastructural micrographs of radioautography performed 30 min after H3 Tdr injection. ×8500.
a Typical lymphoblasts with compact nucleolus and few abundant dense chromatin.
b Ring-shaped nucleolus cell with concentric disposition of granular and fibrillar components around a fibrillar centre, →.
c X-cells with irregularly distributed dense chromatin and compact nucleolus, →.

coated 200 mesh grids. Ilford L4 emulsion is put over the grids with a loop. After a 6–8-week exposition time, grids are developed with 4% Rodinal 8 min, stop bath 30 sec and Acidofix for 10 min. After washing, sections are contrasted with uranyl acetate–lead citrate. At the EM301 Philips examination, about 1000 subcapsular lymphoid cells of each thymus are numerated and the labelling index of lymphoblasts, ring-shaped nucleolus cells and X-cells is calculated.

Results. Half of the whole thymic blast cell population is labelled 15 min after H3 Tdr injection (table). A 'plateau' is reached after 30 min. A similar situation is observed for the lymphoblasts the labelling index of which increases from 58.3 to 73.8% between 15 and 30 min. The small variations observed later on are not statistically significant. More than 90% of X-cells have incorporated the radioactive precursor during the 30 min following the injection. After 2 h, all these X-cells are labelled. The evolution of the ring-shaped nucleolus cell labelling index is quite different: during the first h, only 30% of them have incorporated tritiated thymidine. However, 1 h later on, this labelling index is doubled. At 8 h, the labelling index of the different analyzed cell aspects are very similar and represent about 40%.

Discussion. The labelling index of the whole group of blast cells confirms the numerous investigations which indicate that this population has a high proliferative capacity[5,6]. As the lymphoblasts represent the majority of the thymic blast cells, it is clear that their labelling index has a major influence on the percentage of labelled blasts. Both indexes are similar in our material.

The values obtained for the ring-shaped nucleolus cells and the X-cells are incompatible with the hypothesis that these cells constitute cellular entities which would be able to pass through all cycle phases. Their typical nuclear ultrastructure characterizes probably their place in a definite part of the cycle.

The high labelling index of X-cells implies that these blasts are nearly always in S-phase. On the contrary, the rather low index of ring-shaped nucleolus cells suggests that many blast cells have this peculiar nucleolar structure when they are outside S. However it is impossible at the present time to determine their precise position in the cycle. If we accept the values generally admitted for the duration of the cycle phases in lymphoid blast cells[5,6], many hypotheses can be proposed; only the most likely ones will be discussed.

If ring-shaped nucleolus cells correspond to cells which pass from the end of G_1 to S, a progressive decrease of their labelling index would be expected. If these cells were in the middle of S-phase, their labelling index would stay nearly constant. The increase of the labelling index we have observed between the first and the 2nd h rules out the both possibilities. On the contrary, the variations we have found could be explained if it is assumed that the ring-shaped nucleolus cells are at the end of S and in G_2. This hypothesis would imply that in some blast cells, the duration of G_2 is longer than normally. (In proliferating lymphoid cells, G_2 is estimated to last 45 min.) Our values are also consistent with the position of ring-shaped nucleolus cells from the end of S to the beginning of G_1. This hypothesis could also explain not only the increase of the labelling index, but its doubling between the first and the 2nd h, since many cells would have undergone mitosis.

The cells labelled 8 h after the administration of the radioactive precursor are necessarily derived from those which have incorporated thymidine during the first h. The 43% of X-cells labelled after these 8 h are necessarily in the S-phase of a second cell cycle.

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