pycnotique et des cellules bloquées en métaphase est moins important que chez les individus léthaux dans les tissus correspondants. Au voisinage des ébauches en régression, on observe parfois de nombreux polynucléaires sans toutefois constater de plages de nécrose étendues.

Discussion et conclusions. L'association en parabiose permet la survie, le développement et l'étude ultérieure d'embryons léthaux[6]. Cette technique a été utilisée chez l'Axolotl par HUMPHREY[2,3] pour tenter d'assurer la viabilité d'embryons porteurs de mutations léthales. Cet auteur a pu ainsi montrer que l'expression de la mutation «v» (caractérisée par une vasodilatation générale) est totalement masquée grâce à l'association en parabiose alors que celle de la mutation «r» (caractérisée par un ralentissement de la croissance générale et par des lésions du pronéphros) n'est pas modifiée (plusieurs mois après l'opération, les organes de l'individu léthal sont en cours de dégénérescence).Les résultats que nous présentons montrent qu'il en va de même pour la mutation «léthal-mitotique». L'expression de la mutation dans les associations est la même que chez les individus léthaux libres et la régression progressive des tissus léthaux est achevée au moment où l'autobionte normal se métamorphose. La situation est comparable en ce qui concerne les greffes d'ébauches d'organes.

Nos expériences montrent que la mutation «léthal-mitotique» s'exprime d'une manière autonome au niveau des organes greffés ou des parabiontes. Cependant, l'association avec des embryons sains permet la survie de certains tissus pendant plusieurs mois. Ceci est à opposer aux travaux réalisés chez le Pleurodèle sur la mutation «ascite caudale»[7] qui ont montré que non seulement l'association en parabiose ne guérit pas l'embryon léthal mais encore qu'elle conduit à la mort précoce du couple formé.

Le caractère cellulaire autonome de l'expression de la mutation «léthal-mitotique» et la présence dans tous les tissus léthaux de nombreuses cellules bloquées en métaphase nous ont conduit à rechercher une anomalie éventuelle de l'appareil mitotique (J. Microsc., à paraître)[8].

Summary. The autonomous cellular expression of a recessive lethal mutation, *lm* ('léthal-mitotique'), isolated in the Salamander *Pleurodeles waltlii* Michah. is demonstrated by the way of parabiotic associations and heterotopic grafts.

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Comparison of the Effects of Lithium, β-Phenylethylamine and Tyrosine on *Xenopus* Embryos

Lithium ions have been shown to have morphological effects on a variety of developing systems[1] Lithium influences the morphology of sponges[2] and alters the pattern of tentacle regeneration in hydra[3]. In sea urchin embryos, lithium treatment produces vegetalization and exogastrulation: that is, over-development of endoderm. Since lithium produces similar effects in the amphibian embryo[4], it is possible that it alters control factors common to embryos of different species.

The elucidation of the mechanisms by which an agent effects the balance of early development in a specific and repeatable way might give some indication of the mechanisms which control this balance in normal development. However, although many biochemical differences between normal and lithium-treated embryos have been described[5], the nature of the action of lithium on amphibian and sea-urchin embryos is unknown.

Lithium has been used in the treatment of certain mental diseases, and research in this field suggests that lithium acts via biologically active amines[6]. This led LALLIER[7] to compare the effects of amines and amino acids on the early development of the sea urchin, *Paracentrotus lividus*, with those of lithium. He found that embryos exposed to 10^{-3} *M* tyrosine for 20 h were weakly vegetalized (less than 20% of embryos vegetalized). Under identical conditions, 2.5×10^{-4} *M* β-phenylethylamine had a strong vegetalizing effect (100% of embryos vegetalized). These and similar results led Lallier to suggest that lithium might be acting via amines in the sea urchin embryo. Since lithium produces similar morphological effects in amphibian embryos, it is of interest to establish if these chemicals can produce vegetalizations in this system too. In this paper the effects of lithium chloride, β-phenylethylamine and tyrosine on amphibian embryos are described.

Materials and methods. Embryos of *Xenopus laevis* were obtained by injecting adults with chorionic gonadotrophin (Pregnyl, Welcome), and the jelly was removed chemically by the method of DAWID[8]. Embryos were cultured in 10% Holtfreter saline, pH 7.3, at room temperature (21 °C) and staged according to NIEUWKOOP and FABER[9]. Embryos of either early cleavage (stage 2–4), mid-cleavage (stage 8–9), blastula (stage 8–9) or late blastula (stage 9–$9^1/_2$) stages were used. They were exposed to lithium chloride, tyrosine or β-phenylethylamine (Sigma) for 3 h, washed in 2 changes of 100 ml of 10% Holtfreter saline, and allowed to develop in 10% Holtfreter saline. Some embryos were cultured continuously in the teratogens. Results were scored when the control embryos had reached the neurula stage (stage 17–19), and apply to embryos exposed for 3 h unless otherwise stated.

Results and discussion. A range of abnormalities was found in these experiments. They were classified into 3 main types: exogastrulae without neural structures; embryos in which the neural folds were separated by protruding yolky cells: and neurulae in which the ventral

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region was expanded. These abnormalities could all be the result of an excessive production of endoderm cells, or vegetalization of the embryos. Other abnormalities occurred less frequently, and were usually defects in the neural structures. Double neural tubes, kinked neural tubes or flattened neural tubes were observed in some embryos.

The proportion of embryos affected depended on the length of exposure to the teratogenic agent (3 h or continuous), the concentration of the agent, and the stage at which exposure commenced. Some variation in susceptibility between batches of eggs from different adults was also observed.

Lithium chloride. A preliminary experiment showed that 10^{-1} *M* was a suitable concentration at which to use lithium chloride. 1.5×10^{-1} *M* lithium chloride caused arrested cleavage and abnormal pigment distribution; 2.5×10^{-2} *M* lithium chloride produced very few abnormalities. Using 10^{-1} *M* lithium chloride, 82% of embryos exposed at early cleavage stages were vegetalized, and 69% of embryos exposed at mid-cleavage to early blastula stages were vegetalized. By the late blastula stage, embryos were not affected by a 3 h exposure to lithium. Thus earlier embryos are more susceptible to lithium than later embryos. Continuous exposure to lithium was found to produce disaggregation of the embryos, and this finding will be discussed in more detail elsewhere[10].

Tyrosine. Embryos at the early blastula stage were exposed to 10^{-2} *M* and 10^{-3} *M* tyrosine for 3 h and continuously. No vegetalization of embryos was observed, and thus tyrosine did not produce vegetalization in amphibian embryos at stages when lithium can do so.

β-Phenylethylamine. Exposure to β-phenylethylamine produced a range of abnormalities similar to that produced by lithium. 10^{-2} *M* β-phenylethylamine caused abnormal pigment distribution and arrested blastulae in all embryos by the end of a 3 h treatment. 10^{-3} *M* β-phenylethylamine produced vegetalized embryos: 57% of embryos exposed at early cleavage stages, 48% of embryos exposed at the early blastula stage, and 9% of embryos exposed at the late blastula stage were vegetalized. At this concentration, β-phenylethylamine also produced a large number of degenerated embryos (41% with early cleavage exposure, 28% with late blastula exposure). An additional effect at this concentration was that the ectoderm of embryos appeared to be degenerating. 10^{-4} *M* β-phenylethylamine produced a weaker vegetalizing effect: 30% of embryos exposed at early cleavage stages, and 18% of embryos exposed at the early blastula stage were vegetalized. No degeneration of the ectoderm of these embryos was observed.

These results, like those of LALLIER[7] for the sea urchin embryo, suggest that lithium and β-phenylethylamine can produce a similar effect, vegetalization, in the amphibian embryo. The amphibian embryo appears more susceptible during early cleavage, and is less susceptible to the action of these teratogens by the late blastula stage. Tyrosine, found by LALLIER[7] to be a weak vegetalizing agent for the sea urchin embryo, did not produce abnormalities in *Xenopus* embryos.

Résumé. On a étudié les effets du lithium, de la β-phényléthylamine et de la tyrosine sur le développement embryonnaire de *Xenopus laevis*. La tyrosine n'a pas d'effet, mais le lithium ou la β-phényléthylamine ont végétalisé les embryons s'ils ont été exposés avant le stade blastula.

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Tumor Formation in the Region of Parotid Glands by DMBA

7,12-Dimethylbenz(a)anthracene (DMBA) is a hydrocarbon carcinogen that selectively induces tumors of the mammary glands in rats[1,2]. When larger doses of 7,12-dimethylbenz(a)anthracene are injected i.v., incidence of luekemia is increased in the rat[3,4]. Mammary tumors and luekemia are the two types of malignancies commonly induced by the administration of this carcinogen. We wish to report the formation of a benign tumor possibly of adnexal or parotid gland origin occurring in the rat associated with the intravenous injection of a 7,12-dimethylbenz(a)anthracene.

Male or female Sprague-Dawley rats weighing 125–150 g were used for the induction of tumors. A lipid emulsion of 7,12-dimethylbenz(a)anthracene (Eastman Kodak Co.) was injected in the tail vein of these rats by a modified procedure of HUGGINS et al.[2]. A 1–2% (w/w) DMBA emulsion was prepared by dissolving 50–100 mg of DMBA in 1.5 ml corn oil, using a warm water bath and the Vortex mixer until a clear solution was obtained. To this DMBA solution was added 3.5 ml rat serum and a fine emulsion was achieved by mixing thoroughly in a Vortex mixer. A volume of 0.2 ml emulsion containing 2–4 mg of DMBA was injected into the caudal vein of the rat at ages of 50, 53 and 56 days or 3 times at weekly intervals beginning at the age of 50 days.

Incidence of parotid tumors induced by 7,12-DMBA

No. rats	Rats with cancer	Incidence (%)
52♀	10	19
11♂	6	54

The tumor developed singly on each side of the cheek, located at the parotid glands and measured approximately 2 cm in diameter.

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