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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[10] M. STANISSTREET, in preparation.

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.

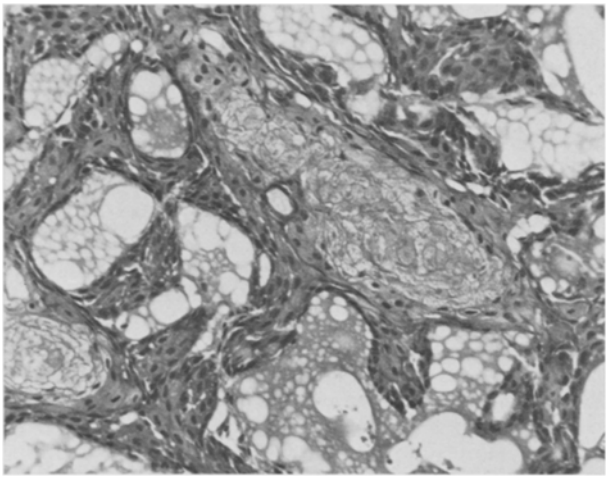
Incidence of parotid tumors induced by 7,12-DMBA

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.

[1] E. FORD and C. HUGGINS, J. exp. Med. *118*, 27 (1963).
[2] C. HUGGINS, L. GRAND and R. FUKUNISHI, Proc. natn. Acad. Sci. USA *51*, 737 (1964).
[3] C. B. HUGGINS and T. SUGIYAMA, Proc. natn. Acad. Sci. USA *55*, 74 (1966).
[4] F. GAL, S. SOMFAI and Z. SZENTIRMAY, Acta haemat. *49*, 281 (1973).

When female rats were injected with 2 mg of 7,12-DMBA 3 times at intervals of 3 days, the majority of them developed mammary tumors; the incidence was higher than 90%. At a dosage of 4 mg/rat at weekly intervals (besides mammary tumors) about 10% of the rats also developed leukemia. Tumors arising in the cheek area were observed in both groups regardless of the dosage, the length of the intervals of injections and developed 2–7 months after the last injection. These neoplasms were histologically benign, and were probably either of adnexal or salivary gland origin, and architecturally resembled sebaceous adenomas to some extent. Histologic morphology was characterized by interconnecting cords of uniform epithelial cells associated with a multifocal central clustering of sebaceous cells (Figure).



Histo-architecture of the tumors from the cheek area most closely resembled sebaceous adenomas. Interconnecting cords of uniform epithelial cells were interspersed by aggregates of foamy sebaceous cells (center). Epithelial cords were further subdivided by small irregular cystic spaces. Hematoxylin and eosin. ×240.

It appeared that the incidence of these neoplasms was higher with male than female rats (Table). Mammary tumors and leukemia rarely developed in the same rat, but simultaneous occurrence of these benign tumors with mammary tumors or with leukemia was not uncommon. The affinity of 7,12-DMBA for certain specific tissues in the induction of tumors has been speculated insofar as its interaction with the nuclear DNA of that particular tissue is concerned[2,3,5]. The method employed in this laboratory in the preparation of the 7,12-DMBA emulsion may facilitate the affinity of this oncogenic material for another region, namely, the salivary gland or adnexa in the cheek area of the rat. The possibility that these benign tumors are unrelated to DMBA cannot be excluded. Spontaneous growth of these tumors, however, is extremely rare. The relative high incidence of these tumors occurring in this study suggests a causal relationship to the administration of DMBA.

Zusammenfassung. Die Injektion von 7,12-Dimethylbenz(a)Anthrazol in die Schwanzvene von Ratten führte zu Bildung von Brusttumoren und in einigen Fällen zu Leukämie, dabei wurde auch, mit grösserer Häufigkeit bei männlichen Ratten, ein neuer Tumortypus im Gebiet der Ohrspeicheldrüse festgestellt.

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[5] C. Huggins, L. Grand and H. Oka, J. exp. Med. *131*, 321 (1970).

[6] Hennepin County General Hospital, Minneapolis, Minnesota 55415, USA.

[7] This work was supported in part by Public Health Service Grant No. CA 14180 from the National Cancer Institute and the Hormel Foundation.

The Blood as an Erythropoietic Organ in Anaemic *Xenopus*

The process of erythropoiesis (blood cell formation) is of general interest since the blood is a rapidly turning-over tissue with a tightly controlled cell population size and a versatile supply system of stem cells. In most vertebrates so far studied the blood cells are replenished by release of more or less well differentiated red blood cells from concentrations of erythropoietic tissue resident in certain body organs. The organ sites of the blood forming tissues vary with the particular animal group and the age of the individual, liver, spleen, kidney and bone marrow being the most frequent locations of erythropoietic tissue[1].

A useful way to study blood cell formation is to accelerate the process by the induction of anaemia although, of course, the animals' response to anaemia may not follow precisely the same pattern as normal erythropoiesis. We are here reporting our observations on the response of the amphibian *Xenopus laevis* to phenylhydrazine-induced anaemia, our main conclusion being that under these conditions most of the process of erythropoiesis is a circulatory phenomenon.

Adult *Xenopus* of both sexes were rendered anaemic by 2 injections of phenylhydrazine, 0.5 ml of a 0.5% solution on 2 successive days. This drug is chiefly active in anaemia induction by dramatically increasing the fragility of the existing erythrocytes, which begin to break up and are removed from circulation within a few days of the first injection. Table I gives the figures for numbers of erythrocytes and other circulating cells before and during recovery from anaemia. It will be seen that within 15 days of the first injection of phenylhydrazine, the blood is essentially devoid of mature erythrocytes. This closely parallels the findings of Grasso[2], in work on induced anaemia in the newt. On different days after the induction of anaemia animals have been bled by heart puncture and the blood cells incubated with nucleic acid precursors. This procedure was performed as previously described[3] except that bovine serum albumin (12 mg/ml)

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[2] J. A. Grasso, J. Cell Sci. *12*, 463 (1973).

[3] N. Maclean, V. A. Hilder and Y. A. Baynes, Cell Differentiation *2*, 261 (1973).