

needed to produce such a hyperpolarization differ by two orders of magnitude.

The decrease in membrane potential observed when the piperazine concentration increases above $4 \times 10^{-3} M$ appears to be due to a non-specific influence of this compound over the entire surface of the muscle cells. The effects of comparable concentrations of GABA have not been investigated, although the shape of curve A suggests that they would exert a similar depolarizing action.

The marked hyperpolarizing action of GABA on *Ascaris* muscle leads one to think that this compound might serve as a basis for the development of new anthelmintics⁴.

Zusammenfassung. Die somatische Muskulatur von *Ascaris lumbricoides* wird durch γ -Aminobuttersäure in Konzentrationen von 10^{-6} – $10^{-5} M$ völlig gehemmt. Eine ähnliche Hemmung wird durch Piperazin ($10^{-3} M$) erzielt.

Beide Substanzen steigern die Cl-Permeabilität der Muskelmembran.

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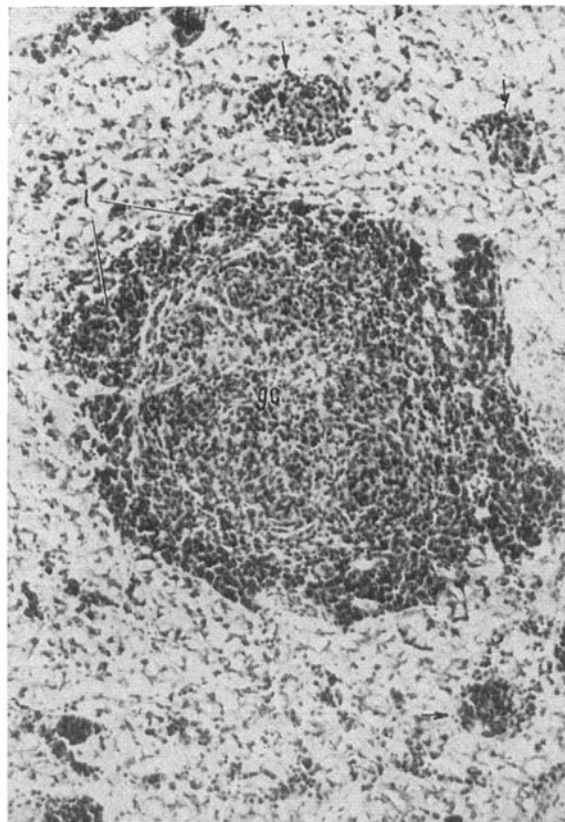
Benzpyrene-Induced Tumours in the Clawed Toad, *Xenopus laevis*

Although a variety of spontaneous neoplasms have been discovered in a large number of anuran species¹, attempts at the chemical induction of tumours in these amphibians have been relatively unsuccessful². Recently, however, it has been shown that the implantation of methylcholanthrene crystals into the South African clawed toad, *Xenopus laevis*³, provokes the development of lymphoid tumours similar to those occurring spontaneously in this species¹; moreover, these tumours are readily transplantable into other *Xenopus*³ or into the urodele species *Triturus cristatus*⁴.

In view of the suggestion of LEONE⁵ that, uniquely in the amphibia, sarcomas develop after methylcholanthrene treatment and carcinomas after benzpyrene application, experiments have been performed in an attempt to induce neoplasia in an anuran species using benzpyrene. Single doses (1.5 mg) of benzpyrene crystals (Roche) were placed in the abdominal cavity of 13 adult *Xenopus laevis laevis* through small cuts in the abdominal wall near the liver, and each wound was closed with a single stitch (Experiment I). Secondly, single small doses of benzpyrene crystals mixed with egg albumen were placed in the dorsal lymph sac or abdominal cavity of 20 immature animals of the same species (Experiment II).

The results of the first experiment are summarized in Table I, which shows that 11 of the 13 animals developed lymphosarcomas between 86 and 288 days after treatment. In 7 of the 11 positive cases the induced tumours affected a multiplicity of visceral organs, notably the liver and kidneys, but in four cases the tumours were less widespread when the animals were killed. The development of lesions in the abdominal wall muscle or skin of five animals is a reflection of the difficulty of introducing the carcinogen without leaving some crystals in the wound area.

The benzpyrene-induced tumours appeared to be histologically very similar to those lymphoid tumours appearing spontaneously in *Xenopus* or induced with methylcholanthrene crystals. A very large nodule some 2 cm in diameter, found in the intestinal mesentery of Case 10, consisted of groups of lymphoid cells separated by connective tissue. Under the abdominal skin of the same ani-



Transfer series case 4. The liver parenchyma lacks the large groups of pigment granules normally found in this organ, but contains a large lymphoid tumour consisting of peripheral small lymphocytes (l) and a 'germinal centre' (gc). Smaller tumour nodules such as those arrowed were spread throughout the liver. H. & E. $\times 200$.

¹ M. BALLS, Cancer Res. 22, 1142 (1962).

² M. BALLS, Nature (London) 196, 1327 (1962).

³ M. BALLS, Cancer Res. 24, 44 (1964).

⁴ M. BALLS, Rev. Suisse Zool. 70, 237 (1963).

⁵ V. LEONE, Tumori 39, 420 (1953).

Table I. Results of Experiment I

Case	Died or killed	Days after treatment	Sex	Diagnosis	Sites affected											
					Liver	Kidneys	Spleen	Muscle	Mesentery	Ovaries	Intestine	Fat-bodies	Skin	Pancreas	Rectum	Oviducts
1	D	15	F	Negative	—	—	—	—	—	—	—	—	—	—	—	—
2	D	86	F	Lymphosarcoma	+	+	+	—	+	+	+	—	—	+	—	—
3	D	114	F	Lymphosarcoma	+	+	+	—	+	—	—	+	—	—	—	+
4	K	150	F	Lymphosarcoma	+	—	—	+	—	+	—	—	+	—	—	—
5	K	161	F	Lymphosarcoma	+	+	+	+	—	—	—	—	+	—	—	—
6	K	248	F	Lymphosarcoma	+	—	—	—	—	—	—	—	—	—	—	—
7	K	260	M	Lymphosarcoma	+	+	+	—	+	—	+	+	—	—	+	—
8	K	270	F	Lymphosarcoma	+	+	—	+	—	—	—	—	—	—	—	—
9	K	275	F	Lymphosarcoma	+	—	—	—	—	—	—	—	—	—	—	—
10	K	276	F	Lymphosarcoma	+	+	+	+	+	—	+	—	—	—	—	—
11	K	277	F	Negative	—	—	—	—	—	—	—	—	—	—	—	—
12	K	277	F	Lymphosarcoma	+	+	—	+	—	+	—	—	—	—	—	—
13	K	288	M	Lymphosarcoma	+	+	—	—	—	—	—	—	—	—	—	—
Total		average 208	—	Lymphosarcomas	11	8	5	5	4	3	3	2	2	1	1	1

Table II. Results of induced tumour transfers

Case	Days after treatment	Dorsal lymph sac		Viscera			
		Persistent growth	Skin invasion	Liver	Spleen	Kidneys	Fat-bodies
1	115	—	—	+	+	+	—
2	115	+	+	+	+	+	+
3	122	—	—	+	+	—	—
4	122	—	—	+	+	+	—
5	122	—	—	+	+	+	—
6	122	+	—	+	+	+	—
Total	average 120	2	1	6	6	5	1

Table III. Results of Experiment II

Case	Days after treatment	Sex	Diagnosis	Sites affected			
				Liver	Kidneys	Spleen	Muscle
1	272	M	Lymphosarcoma	+	+	+	+
2	272	M	Lymphosarcoma	+	—	—	—
3	272	F	Lymphosarcoma	+	+	—	—
4	310	M	Lymphosarcoma	+	+	+	+
5	310	M	Lymphosarcoma	+	—	—	—
6	310	F	Lymphosarcoma	+	+	—	—
7	310	F	Lymphosarcoma	+	+	+	—
8	310	M	Lymphosarcoma	+	+	+	—
9	310	M	Lymphosarcoma	+	+	+	—
10–20	272–310	—	Negative	—	—	—	—
Total	average 297		Lymphosarcomas	9	7	5	2

mal and distant from the wound scar were ten small, well vascularized nodules of lymphoid tissue.

Small fragments of one liver tumour (Case 5) were washed several times in sterilized physiological solution and placed in the dorsal lymph sac of six immature *Xenopus laevis laevis*. The results from this experiment are summarized in Table II and clearly indicate that the benzpyrene-induced tumour was readily transplantable, advanced tumours being found in the kidneys, liver (Figure) and spleen.

From Table III it will be seen that, while Experiment II was less successful than Experiment I, 9 of the 20 treated animals bore lymphoid tumours of liver and of kidneys, spleen or abdominal wall muscle when they were killed after 272 to 310 days.

As with methylcholanthrene a considerable time was necessary between crystal implantation and the appearance of certain tumours. This long latent period may explain the failure of two previous experiments^{6,7} using benzpyrene with anuran amphibians, in which all the treated animals died within a short time.

From the above experiments and others described elsewhere² it is clear that both benzpyrene and methyl-

cholanthrene lead to the development of transplantable lymphoid sarcomas when placed in the anuran amphibian, *Xenopus laevis laevis*⁸.

Résumé. Quelques cristaux de benzopyrène furent implantés dans trente-trois amphibiens anoures (*Xenopus laevis laevis*), vingt d'entre eux montrèrent des lymphosarcomes à différents endroits, le plus souvent localisés dans le foie et les reins. Ces tumeurs induites furent transplantées à nouveau avec succès¹.

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Station de Zoologie expérimentale, Université de Genève, Chêne-Bougeries, Genève (Switzerland), August 14, 1963.

⁶ F. DURAN-REYNALS, Yale J. Biol. Med. 11, 613 (1939).

⁷ J. SKAPIER, Acta Un. int. Cancr. 6, 65 (1948-50).

⁸ This work was supported by the Fonds national suisse pour la recherche scientifique (No. 2219). The author wishes to thank Profs. M. FISCHBERG and A. W. BLACKLER for their advice.

Immunoelectrophoresis of Avian Lens Proteins

While in a previous paper the relationships of *Afropavo* within the Galliformes were studied, both by electrophoretic and immunoelectrophoretic methods¹, the present work deals with a condensed general review of some different immunoelectrophoretic patterns, obtained from the representatives of some phylogenetically important birds.

The lenses were homogenized in *aqua dest.* (200 mg fresh tissue per ml). After centrifugation (20,000 × g for 20 min) the clear supernatant was used for electrophoretic and immunoelectrophoretic examination. Electrophoresis was carried out as described earlier² and the relative mobility of the fractions was determined using the known mobility of a test substance³.

We used antiserum against the soluble proteins of starling lenses (*Sturnus vulgaris*). Combined electrophoresis and immunoelectrophoresis were performed according to RABAEY⁴ on the same plate, which enables exact correlation of the two patterns. All plates are reproduced on the same scale, thus being directly comparable.

The lens antigens of the birds examined were tested with anti-starling antiserum, and thus it may be evident that all immunological patterns can only reflect relationships with the Passeriformes. Some species, however, reacting with the anti-starling antibodies, show a resembling or even similar pattern among each other. Consequently some conclusions about the mutual relationships can be drawn.

Among the several patterns discerned, the one in which most antigens give an individual precipitation line is the most widespread. To this type belong all orders in which the typical song bird component (rel. mob. 0.60 or 0.50) occurs², namely the Ciconiiformes, Falconiformes (*Accipiter nisus*, Figure 1), Strigiformes, Gruiformes, Psittaciformes, Coraciiformes, Piciformes and Passeriformes. It is also the type of the Charadriiformes, Procellariiformes

and Columbiformes, and finally also of the Galliformes, Rheiformes and Casuariiformes.

In a second type, the precipitation lines of several antigens form one single stretched and sharp-cut line. This is the type of the Anseriformes, Podicipediformes and Gaviiformes, and also of *Alca torda* and *Cochlearius cochlearius*, which is represented in Figure 2.

Thirdly, the rather simple electrophoretic runs of the Sphenisciformes, the Phalacrocoracidae-Sulidae (*Sula basana*, Figure 3) and of *Uria aalge* show a simple but characteristic immunological reaction.

Among the birds with rather aberrant patterns, we would cite *Cuculus canorus*, where in spite of an apparently reduced electrophoretic pattern, the immunological reaction shows more well developed and sometimes sharp-cut precipitation lines.

A discussion of all patterns and their similarities in detail would lead us too far, but meanwhile it is clear that the immunological results confirm a lot of conclusions already drawn from the electrophoretic runs.

At first the resemblances between all Passeriform lens pherograms (showing the typical 0.60 fraction) are accentuated by their analogous precipitation patterns in immunoelectrophoresis.

Secondly, some presumed relationships between the birds with an Anseriform pattern could be confirmed (e.g. grebes-loons-auks), while also new perspectives are opened (ducks-*Cochlearius*-herons).

Finally there is no doubt that all birds with a Sphenisciform pattern are pretty closely related. The rather astonishing position of *Uria aalge* is confirmed by the absence

¹ H. GYSELS and M. RABAEY, Bull. Soc. zool. Anvers 26, 72 (1963).

² H. GYSELS, Exper. 19, 107 (1963).

³ M. RABAEY and G. VERRIEST, Ann. Soc. zool. Belg. 88, 373 (1958).

⁴ M. RABAEY, Exp. Eye Res. 1, 310 (1963).