formate-C^{14}. The use of other radioactive precursors of purine nucleotides to measure these effects is limited by the dilution incurred by the non-radioactive pools formed from the AIC. The specific radioactivity of both polynucleotide guanine and adenine following administration of formate-C^{14} in the presence of 30 mg of AIC · HCl per kg was comparable to that observed with 1.25 mg of AIC · HCl per kg, a finding which suggested that increased levels of AIC did not result in greater incorporation of this molecule into the polynucleotide fraction. Although considerable variation was obtained in cells from mice treated with chloropurine, the data indicate that increased concentrations of AIC decreased the chloropurine-induced inhibition of polynucleotide guanine formation.

Although no definitive explanation of these results can be given, a number of possibilities exist. One is that exposure of these cells to large quantities of AIC results in an increase in the metabolic pool of inosine 5′-phosphate, a metabolite which serves to protect the enzyme inosine 5′-phosphate dehydrogenase from the inhibitory effects of the nucleotide of chloropurine[7]. That increasing levels of AIC did not increase the labeling of polynucleotide guanine by formate-C^{14} would suggest that intracellular control mechanisms maintain the relative size of precursor pools of guanine nucleotides at a relatively constant level in the presence of a large amount of AIC in cells not exposed to chloropurine; however, it is possible that in the presence of available AIC, significant increases in the intracellular concentration of inosine 5′-phosphate did occur in cells treated with the purine analogue. Alternatively, AIC may decrease chloropurine-induced inhibition of guanine nucleotide synthesis either by competing with chloropurine for the available supplies of phosphoribosylpyrophosphate, thereby limiting the amount of the active inhibitory form (i.e., chloropurine ribonucleoside 5′-phosphate) present at the enzymatic site or by competing for transport. A decision between these alternatives must await further evidence. Nevertheless, the results obtained support the concept that the blockade of inosine 5′-phosphate dehydrogenase activity by 6-chloropurine is associated with the ability of this compound to enhance the antineoplastic properties of the glutamine antagonist azaserine[8].

Zusammenfassung. 4-Amino-5-imidazolcarboxamid hat eine antagonistische Wirkung auf die synergistische Tumorhemmung, die sich aus der gemeinsamen Anwendung von 6-Chlorpurin und Azaserin ergibt. Dies steht im Einklang mit der Fähigkeit des 4-Amino-5-imidazolcarboxamids, auch die Hemmung des Einbaues von C^{14}-Format in das Guanin von Polynucleotiden, durch 6-Chlorpurin hervorgerufen, teilweise zu unterbinden.

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On the Loss of Mesodermal Competence of the *Triturus* Gastrula Ectoderm in vivo

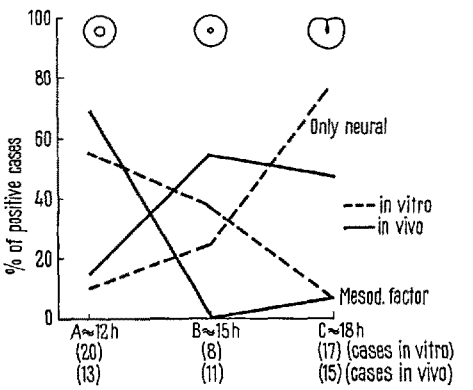
In an earlier work[1] I was able to show, by cultivating pieces of ectoderm of young *Triturus vulgaris* gastrulae as isolated vesicles for various lengths of time and then inserting a heterogeneous inductor (kidney or bone marrow) into these explants, that the mesodermal competence, i.e. the capacity to react to mesodermalizing stimuli, is lost earlier than the neural competence. The present experiments were carried out in order to see whether the intact ectoderm of an old gastrula reacts to the inducing influence of bone marrow in a manner different from that of isolated ectoderm of corresponding age.

Material and methods. For all operations the sandwich technique was used. Small pieces of alcohol-fixed (70% alcohol for 3–48 h in a refrigerator) bone marrow from the femur of young male guinea-pigs were used as inductors. After 8–10 days of cultivation in Holtfreter saline with a double phosphate buffer[2], the explants were fixed in Bouin, stained and histologically examined.

The age of the donor gastrulae was determined partly in hours, taking as starting point Harrison stage 10+ (blastopore straight or slightly curved), partly from the developmental stage only. The explants were divided into four series as follows: A, 10–12 h (Harrison stage 12); B, 13–15 h (Harrison stage $12^1/_2$); C, 16–18 h (Harrison stage 13); D, 20–24 h (Harrison stage 14–15).

Results and discussion. The results are summarized in the Table.

Of the 24 control explants without an inductor (6 for series A, 10 for B, 5 for C, and 3 for D), only one (D) showed any inductions: an archencephalon, evidently



The differential loss of the neural and mesodermal competence of *Triturus* gastrula ectoderm in vitro[1] and in vivo. Mesod. factor: cases with spinocaudal and/or deuterencephalic inductions. Only neural: cases with only archencephalic or unspecific neural (neuroid) inductions.

[1] A. LEIKOLA, Ann. Zool. Soc. 'Vanamo' *25*, 2 (1963).
[2] E. M. DEUCHAR, J. exp. Biol. *30*, 18 (1953).

The different inductions obtained with bone marrow in different phases of competence, expressed as percentages of all available cases (% total) as well as percentages of all positive (mesenchymal, mesodermal, and neural) cases (% pos.)

	A			B			C			D	
	Cases	% total	% positive	Cases	% total	% positive	Cases	% total	% positive	Cases	% total
Total	14	100		24	100		31	100		13	100
Atyp. epidermis	14	100		23	96		31	100		13	100
Norm. epidermis	11	79		15	63		17	55		2	15
Positive	13	93	100	11	46	100	15	48	100	(2)	(15)
Mesenchyme	9	64	69	2	8	18	7	23	47	(2)	(15)
Melanophores	9	64	69	10	42	91	11	35	73	–	–
Mesodermal	7	50	54	–	–	–	1	3	7	–	–
Notochord	4	29	31	–	–	–	1	3	7	–	–
Muscle	3	21	23	–	–	–	1	3	7	–	–
Fin	6	43	46	–	–	–	–	–	–	–	–
Neural	10	71	77	6	25	55	8	26	53	–	–
Spinal cord	7	50	54	–	–	–	1	3	7	–	–
Deuterencephalic	2	14	15	–	–	–	–	–	–	–	–
Archencephalic	–	–	–	3	13	27	2	6	13	–	–
Neuroid	2	14	15	3	13	27	5	16	33	–	–

owing to an error in experimental technique. No inductions occurred in those cases where the implant was not found.

The results indicate that after about 12 h the mesodermal competence of the ectoderm is for the most part lost and only the neural competence, itself reduced, is left until early neurula stage. It would seem that in vivo the competences tended to be lost slightly earlier than in vitro[1]. What is more important is the occurrence of the same competence phases in the same sequence. This would suggest that the loss of competence is an autonomous process not remarkably influenced by the neighbouring tissues in the intact gastrula.

Contrary to the results of TSENG[3], who used the same method but a different species, *Cynops orientalis*, no shift towards more ventral mesodermal structures during the loss of competence was found in the present experiments, which is more in accordance with the work of GEBHARDT and NIEUWKOOP[4] with lithium-treated *Ambystoma* ectoderm, as well as with my own experiments with *Triturus* ectoderm in vitro. More work is certainly needed before we have a clear picture of the response of the aging ectoderm to mesodermal induction.

Résumé. Des expériences ont été effectuées pour déterminer l'action inductrice de la moelle osseuse sur l'ectoderme de la gastrule agée de *Triturus vulgaris*. Un Tableau et une Figure en présentent les résultats.

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Department of Physiological Zoology, University of Helsinki (Finland), February 28, 1965.

[3] M.-P. TSENG, Acta biol. exp. sinica *8*, 230 (1963).

[4] D. O. E. GEBHARDT and P. D. NIEUWKOOP, J. Embryol. exp. Morph. *12*, 317 (1964).

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Responses Evoked by Stimulation of the Acoustic Pathway During the Sleep-Wakefulness Cycle[1]

Since thalamic transmission and cortical responsiveness are strictly related to the level of vigilance in both somatic[2-4] and visual[5,6] sensory systems, an analysis of possible functional changes occurring in the acoustic pathway during the sleep-wakefulness cycle has been undertaken. 14 adult cats carrying electrodes chronically implanted into the inferior quadrigeminal brachium (IQB), acoustic radiations (AR), and over middle and posterior ectosylvian giri (primary auditory areas) have been used. For detailed technical information regarding the exact location of the electrodes, the technique used within the experimental sessions and the statistical evaluation of results, the reader is referred to DAGNINO et al.[7,8].

Results. A. (Figure 1) The postsynaptic components of the cortical response evoked by stimulation of IQB (a positive-negative deflection, initial latency 2.0–3.5 msec) showed, on arousal from light sleep, a significant amplification in 65% of the trials. An even greater and more constant (90%) increase in amplitude occurred when the animal fell into deep sleep, while on subsequent arousal