recovery in the period 15–25 days thereafter remained at an insignificant level (figure 2). Labelled stage 2 cells were initially in much lower numbers but the pattern of change subsequently showed a similar trend to that of labelled stage 1 cells. On the other hand, stages 3 and 4 cells showed an almost reciprocal graph of labelling to stage 1 cells, i.e., the incidence of labelling was insignificant for the 1st 15 days but rose dramatically thereafter and was relatively constant in the period 25–60 days (figure 2). When the mean isotope grain counts for mast cells of the various ages were analyzed it was found that the isotope concentration (mean grain count) for stages 1 and 2 cells was initially high but dropped rapidly to one half and one quarter of the initial means by 4 and 8 days respectively. Labelled stages 3 and 4 cells appeared first at 15 days and have a relatively low mean grain count level approximating that of stage 1 cells at 15 days. Labelling of low intensity thereafter rose to a plateau in the period 30–60 days. Similar findings were recorded for mast cells in mesenteric and omental windows. In conclusion, it would seem that the relatively rapid drop in labelled stage 1 cells and the rise in the number of labelled stages 3 and 4 cells with diluted isotope content following ^{3}H-thymidine administration at birth indicates that stage 1 and to a lesser extent stage 2 mast cells comprise a mitotic pool of cells whereas stages 3 and 4 cells constitute a maturational pool[9].

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Effects of angiotensin II and of an angiotensin II receptor antagonist on Simian virus 40-induced tumor growth in vivo

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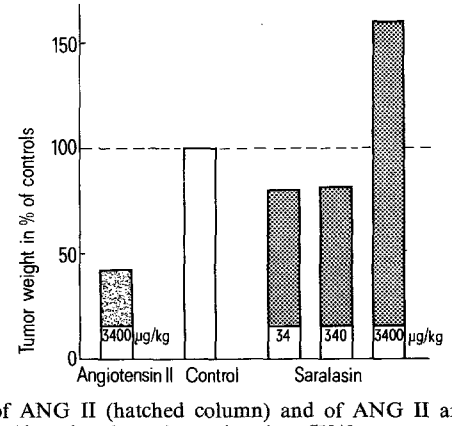
Summary. The effects of angiotensin II and of the competitive angiotensin II receptor antagonist saralasin on in vivo tumor growth were investigated in hamsters. Angiotensin II strongly inhibited tumor growth while saralasin stimulated it, though the high dose used had partial agonistic angiotensin II-like actions. Lower doses of saralasin were without significant effect on tumor weights.

We have recently shown that angiotensin II (ANG II) and the ANG II antagonist [sar[1], val[5], ala[8]] ANG II (saralasin) affect cell growth and intracellular renin concentration in an established cultured cell line of 3T3 mouse fibroblasts, and in their fast growing Simian virus 40 (SV40) transformed counterparts[3]. The competitive ANG II antagonist saralasin inhibited cell proliferation in 3T3 and SV3T3 cells almost completely, and increased intracellular renin content. ANG II, in contrast, stimulated cell growth in 3T3 and slightly diminished cell proliferation in SV3T3 cells. These in vitro findings led us to investigate the effect of ANG II and of saralasin on the growth of SV40 induced tumors in vivo. Our studies were also prompted by the evidence that ANG II stimulates the growth of adrenal gland cells[4] and influences biochemical events which are involved in growth control such as cyclic nucleotide, DNA, RNA and protein synthesis[4,5].

Materials and methods. For tumor induction[6], newborn Syrian hamsters were inoculated s.c. in their neck within 24 h after birth with 0.1 ml of crude virus (10^6–10^7 PFU)[7]. 3–4 weeks after injection, tumors were detected as s.c. nodules at the site of application. Another 2 weeks later, the animals were killed and the tumor was transplanted to adult hamsters[6]. ANG II and saralasin respectively were applied daily at 10.00 h s.c. in 0.1 ml of oil, beginning 1 day after tumor transplantation. The drugs were suspended freshly each time by ultrasonification. ANG II was injected at a dose of 3400 µg/kg b.wt. Saralasin was administered at doses of 3400, 340 and 34 µg/kg. Controls (CO) received 0.1 ml oil only. The experiments were terminated when the sarcomas were well developed and before the tumor tissue became necrotic. The hamsters were bled by cutting the carotid arteries, blood was collected on ice in plastic tubes containing an angiotensinase inhibitor cocktail[8] and centrifuged at 4 °C. The plasma was stored at −30 °C. Adrenal glands were excised and prepared for morphometric studies. Small tissue samples were taken from tumor, kidney, brain, heart, gut, muscle, skin,



Effects of ANG II (hatched column) and of ANG II antagonist saralasin (dotted columns) on in vivo SV40 tumor growth in hamsters expressed in percentages of controls (open column); each group consisted of 10 or more animals.

spleen and liver respectively for histological examination. In a first series of experiments, 10 hamsters were treated with 3400 µg/kg saralasin daily and 10 hamsters treated with oil served as controls. The animals were killed after 16 days. In a 2nd experiment, 60 hamsters were divided into groups of 15 animals. The 1st group served as control, the 2nd group received 3400 µg/kg ANG II, whilst the 3rd and 4th group were treated with 340 µg/kg saralasin and 34 µg/kg saralasin respectively. The animals were killed after 2 weeks.

Tissue and plasma renin measurements were performed as has been described elsewhere[8]. Briefly, the left kidney was homogenized in 3 ml of TRIS-maleate buffer pH 6.5, then frozen and thawed 3 times. After centrifugation at 10,000 × g, 1 ml aliquots of the supernatant were diluted 1:400 and incubated with 50 mg of rat substrate at pH 6.5 in the presence of DOWEX 50W × 2 NH_4^+ during 1 h in a final volume of 3 ml. Incubation of 0.2 ml of plasma was done under the same conditions during 2 h. Between 200 and 800 mg of tumor tissue were prepared as mentioned above except that homogenization and incubation were performed in TRIS-maleate buffer pH 5.5. Tumor tissue was incubated with 50 mg of dog substrate during 4 h. Results are expressed as means ± SEM. Statistical significance of differences was calculated by Student's unpaired t-test.

Results and discussion. The histology of all organs examined confirmed the SV40 tumor to be a sarcoma of spindle-shaped cells without metastases[9]. Administration of 3400 µg/kg saralasin accelerated tumor growth and diminished tumor renin in hamsters as compared to sham treated controls (table). Renin levels in kidney and plasma were strongly reduced by saralasin. Enzyme concentrations in the kidney were 6.17 ± 1.17 µg ANG I/g/h (saralasin) and 219.12 ± 25.71 µg ANG I/g/h (controls) respectively ($p < 0.001$). The following renin concentrations were measured in plasma: 0.52 ± 0.08 ng ANG I/ml/h in saralasin treated animals and 7.09 ± 0.98 ng ANG I/ml/h in controls ($p < 0.001$). The zona glomerulosa of the adrenal gland was widened 1.6 times under drug application, whilst the zona fasciculata and reticularis remained unchanged in treated and untreated animals.

These results indicated that the ANG II antagonist saralasin applied at 3400 µg/kg per day probably exerted agonistic ANG II-like effects since plasma and kidney renin as well as tumor renin concentrations were decreased. The hypertrophy of the adrenal gland, indicating a stimulatory effect of saralasin on steroid synthesis, supported this view. Such an agonistic influence of ANG II receptor antagonists has been reported[10,11].

We have therefore treated a 2nd series of hamsters with a high dose of ANG II and with smaller amounts of saralasin. The smaller doses of saralasin did not significantly affect tumor weight, while ANG II markedly inhibited tumor growth (table). No significant difference in tumor renin concentration was observed between controls and treated animals (table). The tumors in the 2nd experimental series of hamsters grew faster, while their renin content was lower (table). Since the same strain of animals was used, these differences between experiment 1 and experiment 2 may be related to the tumor induction and tumor transplantation which have to be done de novo for each experiment[6,7]. If the results were therefore expressed as percent change compared to controls it became evident that 3400 µg/kg ANG II diminished tumor weight by about 57% and that on the other hand 3400 µg/kg saralasin stimulated tumor growth by about 60%; lower doses of saralasin tended to decrease tumor weight by 18% (340 µg/kg) and 20% (34 µg/kg) respectively (figure), but these changes were statistically not significant.

We have previously reported that in vitro, saralasin inhibited cell growth in 3T3 and SV3T3 cells[3]. In vivo we now observed an increase of SV40 tumor growth in hamsters at the highest dose and no significant effects at lower doses of saralasin. It is doubtful whether this discrepancy between in vivo and in vitro effects can be solely related to the agonistic ANG II-like activities of saralasin at high doses, since ANG II at the same high amount had just the reverse effect of saralasin, i.e. inhibition of tumor growth in vivo. The smaller doses of saralasin influenced neither tumor growth nor tumor renin content significantly. In vitro ANG II increased cell proliferation in 3T3 cells but slightly diminished it in SV3T3 cells[3]; this is compatible with the inhibitory effect of ANG II on tumor growth in vivo.

The administration of ANG II and saralasin respectively has systemic effects influencing various hormonal systems and blood pressure. No steroid measurements were performed in these experiments. However, mineralocorticoid synthesis and release can be assumed to be increased in the ANG II treated hamsters and simultaneous stimulation of glucocorticoids is also possible[12]. The high dose of saralasin probably had an agonistic ANG II-like effect on steroidogenesis too, since the zona glomerulosa of the adrenal was hypertrophied. In some types of oncogenic cells such as glioma or leukemia cells, glucocorticoids have been reported to inhibit cell growth[13,14]. On the other hand they may increase cell proliferation in 3T3 cells[15,16]. Growth of SV40 transformed 3T3 cells seems not to be stimulated[17].

In conclusion, ANG II decreased SV40 tumor growth in vivo, while the ANG II antagonist saralasin at a high dose had the reverse effect. These in vivo results are in contrast to in vitro findings where saralasin strongly inhibited cell proliferation. The different actions in vivo and in vitro cannot be explained at present. The control of, and the pharmacological interference with cell growth in vivo is much more complex, however, than under standardized conditions in vitro. A possible relation to other hormonal systems, e.g. adrenal gland steroids, needs to be investigated.

	Controls	ANG II 3400 µg/kg	Saralasin 34 µg/kg	340 µg/kg	3400 µg/kg
Tumor weights in g	2.56 ± 0.47				4.10 ± 0.42*
Tumor renin in ngANG I/g/h (1st series)	44.49 ± 3.77				31.03 ± 2.49*
Tumor weights in g	13.36 ± 1.43	5.70 ± 0.65***	10.74 ± 0.99	10.96 ± 1.54	
Tumor renin in ngANG I/g/h (2nd series)	17.23 ± 2.28	17.77 ± 3.25	12.50 ± 2.38	14.98 ± 2.65	

Tumor weights and tumor renin content in hamsters treated with ANG II and with saralasin respectively compared with the appropriate controls; * $p < 0.05$, *** $p < 0.001$; $n = 10$ or more.

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Scanning electron microscopy of cells from hydroxyurea-arrested blastulae of *Xenopus laevis*

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Summary. Cells from *Xenopus* embryos blocked at the blastula stage by treatment with hydroxyurea have been isolated and cultured in vitro. The morphology of these cells has been compared with that of cells from normal embryos using scanning electron microscopy. Cells from such hydroxyurea-blocked embryos do not show the features, or changes in features, in culture shown by cells from normal embryos.

Recently the properties of cells isolated from amphibian early embryos have been studied in the hope that they might provide information on the mechanisms involved in normal morphogenesis. The types of cell movement shown in vitro, and the proportions of cells exhibiting movement vary with the stage of the embryo from which the cells are taken, and are different for different germ layers[2,3]. Similarly, work in our laboratory has shown that the scanning electron microscopical appearance of cells isolated and cultured on glass differ between cells from different embryonic tissues[4], and is dependent upon the stage of the embryo providing the cells[5]. These findings are consistent with the idea that the properties shown by cells in vitro are significant to normal morphogenesis. In addition, cells from genetically deficient hybrid embryos which do not gastrulate, or gastrulate abnormally, show altered motility in vitro[6].

Some years ago it was shown that treatment of embryos of echinoderms and amphibians with hydroxyurea blocked development at the 4–8-cell stage and late blastula stage

Percentages of cells from normal and hydroxyurea-blocked blastulae of *Xenopus* showing different features by scanning electron microscopy

		Time in culture	Total number of cells	% of cells in each condition							
				Filopodia	Few filopodia	Flattened	Pseudopodia	Elongated	Webbing	Featureless	'Blebbing'
Ectoderm	Control	3 min	228	48	35	3	–	–	–	14	–
		0.5 h	280	–	7	82	–	–	–	11	–
		3 h	258	–	–	37	37	–	–	26	–
	Cells from hydroxyurea-blocked blastulae	3 min	295	–	–	7	–	–	11	82	–
		0.5 h	228	–	–	3	–	–	4	93	–
		3 h	180	–	–	6	–	–	–	94	–
	Cells cultured in presence of hydroxyurea	3 min	245	–	–	3	–	9	–	23	65
		0.5 h	296	–	3	33	–	5	8	40	11
		3 h	229	–	–	–	–	–	–	94	6
Endoderm	Control	3 min	108	–	–	5	–	–	12	83	–
		0.5 h	145	–	3	39	2	6	–	50	–
		3 h	75	–	–	34	22	12	–	32	–
	Cells from hydroxyurea-blocked blastulae	3 min	213	–	6	5	–	1	3	85	–
		0.5 h	176	–	–	5	–	3	5	87	–
		3 h	217	–	–	10	–	3	–	87	–