A combined histochemical and autoradiographic study of the distribution and maturation of peritoneal mast cells in the rat

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Summary. The relationship of mitotic activity and degree of cytological differentiation for mast cells of the peritoneal cavity of the rat was studied using combined autoradiographic and histochemical techniques. A mitotic pool of mast cells can be differentiated from a non-mitotic pool, the former containing cells with immature cytoplasmic differentiation and the latter with fully developed cytoplasmic histochemical properties.

Mast cells are scarce in the peritoneal cavity of the newborn rat[1] but plentiful in the adult animal[2]. By means of histochemical staining of their granules with alcian blue/safranin mast cells exhibit 4 stages of maturation, Stage 1 being the most immature and stage 4 being fully mature, and it has been reported that while the adult rat contains many peritoneal mast cells of all stages of maturation the newborn and immature rat have only a few mast cells of stages 1 and 2 of development[1,2]. The transition from the neonatal distribution to the adult distribution of mast cells for maturation and concentration occurs within a period of 30 postnatal days. It is not known whether this transition is achieved dominantly by the proliferation of mast cells in situ or by heteroplastic differentiation of precursors, nor is it known when mast cells cease mitotic activity and commence the process of cytoplasmic maturation.

In an attempt to provide information on these questions, simultaneous histochemical determination of stage of maturation and autoradiographic analyses of mitotic activity was carried out for mast cells in peritoneal smears and on omental and mesenteric spreads in rats aged 0.5–60 days, as well as in adult animals. 6 adult Albino Wistar rats (180–220 g) were given a single dose of ^{3}H-thymidine i.v. (1–2 μCi/g b.wt) 1.5 h before sacrifice and batches of 4 neonatal rats each from different litters ranging in age from 0.5–60 days were given ^{3}H-thymidine s.c. (1–2 μCi/g b.wt), 1.5–2 h before sacrifice. Peritoneal smears as well as mesenteric and omental spreads were fixed in Newcomer's fluid[3] and stained with alcian blue/Safranin[2]. For autoradiographs, stained slides of tissue preparations were first coated by dipping in a 1% solution of celloidon in ether/ethanol and then coated with Ilford K5 Nuclear Emulsion and stored vertically for 5 weeks in the dark. The emulsion was then developed in Kodak D19, and fixed with Ilford Hypam Rapid Fixer. After washing in water and hardening in 4% formalin, preparations were washed, cleared in xylol and mounted in DPX.

For adult rats, of a total of 4000 peritoneal mast cells counted, only 6 stage 1 and 1 stage 2 mast cells were isotopically labelled. Of some 12,000 mast cells counted in mesenteric and omental preparations only 2 stage 1 and 1 stage 2 cells were found to be isotopically labelled. No stages 3 or 4 mast cells were found to have been labelled. These results confirm previous evidence of the extremely low incidence of mitotic activity of mast cells in the adult animal[4–8].

Peritoneal mast cells in the neonatal rat are however mitotically active. Figure 1 illustrates the percentage of isotopically labelled mast cells of each of the 4 stages of maturation in the free peritoneal fluid of neonatal rats. The majority of labelled cells were in stages 1 and 2 of maturation although after the 4th postnatal day stages 3 and 4 mast cells also took up the radioactive label (figure 1). Similar results were obtained for mesentery and omentum.

In further experiments all newborn rats from several litters were given either a single dose of isotope s.c. or 4 doses over a 24-h-period and batches of 4 rats from different litters were sacrificed from 2 to 60 days. Autoradiographs were again prepared for free peritoneal cells, mesentery and omentum. In addition to enumerating the percentage of labelled mast cells, the mean isotope grain count was recorded for labelled cells at the various age parameters. Results were essentially identical for rats given a single or 4 doses of isotope. For free peritoneal mast cells the initial high percentage of labelling for stage 1 cells declined dramatically over the 1st 14 days and apart from a slight

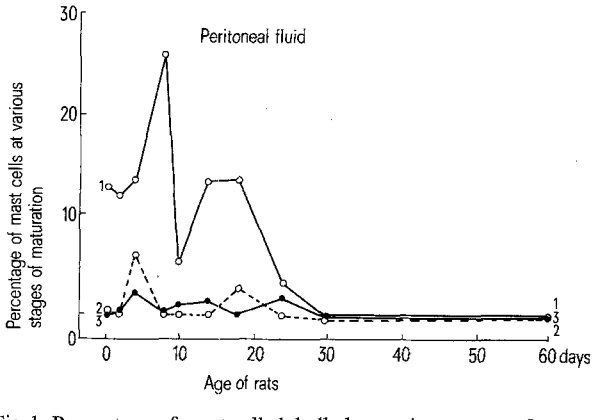

Fig. 1. Percentage of mast cells labelled at various stages of maturation in peritoneal fluid of rats aged 0.5–60 days following a single dose of ^{3}H-thymidine. The response line for stage 4 cells is not shown. Only 2.8% of stage 4 cells are labelled on day 10. Each point on the graph represents the mean from 4 rats. Numbers on each side of the response lines indicate the stage of maturation of mast cells.

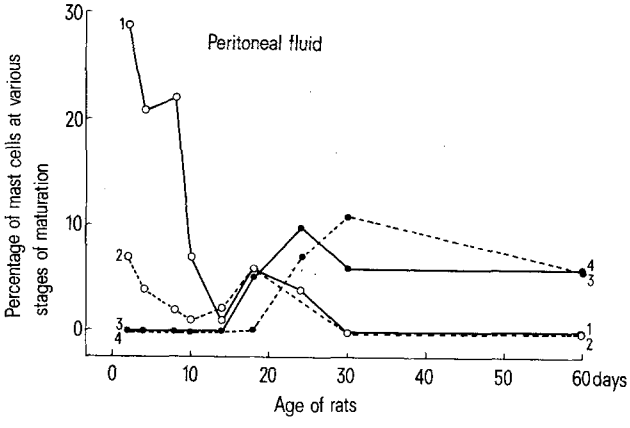

Fig. 2. Percentage of mast cells labelled at progressive stages of maturation from peritoneal fluid of rats at various age intervals from 0.5 to 60 days following a single dose of ^{3}H-thymidine at birth. Results were similar for rats given 4 doses of ^{3}H-thymidine over 24 h. Each point on the graph represents the mean from 4 rats. Numbers on each side of the response lines indicate the stage of maturation of mast cells.

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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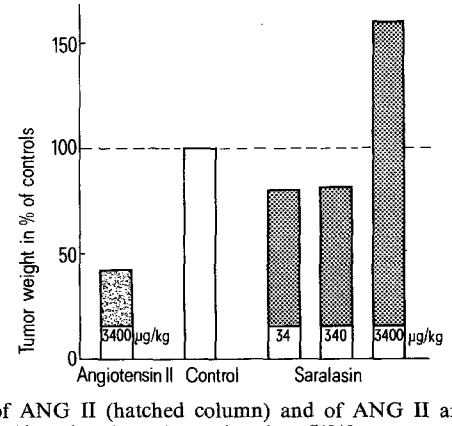
Summary. The effects of angiotensin II and of the competitive angiotensin II receptor antagonist saralasin on in vivo tumor growth were investigated in hamsters. Angiotensin II strongly inhibited tumor growth while saralasin stimulated it, though the high dose used had partial agonistic angiotensin II-like 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.