The Effect of Bovine Seminal Ribonuclease (AS RNase) on Cells of Crocker Tumour in Mice

The aspermatogenic and antiembryonic effects of bull seminal vesicle fluid[1-4] were recently specified in the protein substance activity of this fluid[5,6]. This substance was isolated[7] and its ribonuclease degradation activity was determined[8]. The designation AS RNase was proposed for this bull seminal ribonuclease. When comparing the substrate specificity of AS RNase with the bovine pancreatic RNase A, it was found that both ribonucleases have practically identical effectiveness[9]. It was, however, detected that AS RNase differs from pancreatic RNase A in its biological effect as demonstrated by the capacity to affect cytostatically testicular and embryonic tissue in mice[8] and other animal species[10]. In this paper the effect of AS RNase on the cells of Crocker tumour in mice is dealt with.

Materials and methods. As a source of AS RNase, the partially purified and lyophilized substance of the bull seminal vesicle fluid, designated BF[7] and containing 65–70% pure AS RNase, was used. In all the experimental groups of mice, this substance was dissolved in sodium citrate (29 g $Na_3C_6H_5O_7.2H_2O$ in 1000 ml H_2O, pH 6.7) in the amount of 10 mg/ml (7 mg of pure AS RNase/ml). Sodium citrate without AS RNase was administered to control groups of animals.

This basic AS RNase solution was applied to mice with Crocker tumours in the following way: a) homogenate of Crocker tumour was incubated with the basic AS RNase solution in the volume ratio 1:2. After 1 h incubation this mixture in the amount of 0.25 ml per mouse was injected intracutaneously in the neck region in 16 mice of the H strain. Second part of the homogenate was incubated for 1 h with sodium citrate and injected in a similar to the control group of mice. In a further experiment (b) the AS RNase solution, after 1 h incubation with Crocker tumour homogenate, was centrifuged, the supernatant replaced by sodium citrate without AS RNase and the resulting suspension retransplanted to 20 mice. In the experiment (c) AS RNase in the amount of 0.7 mg in

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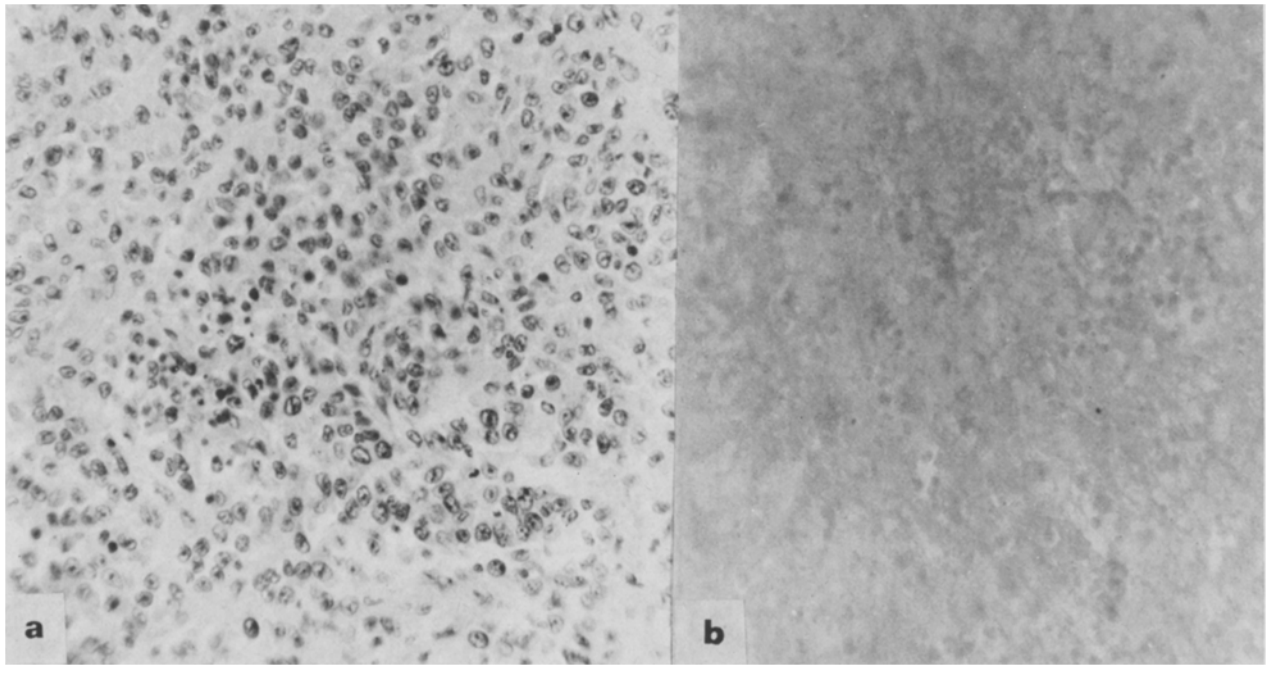
The AS RNase effect on Crocker tumour cells in mice

Mode of AS RNase administration and term of planned killing	Experimental group				Control group			
	No. of mice injected with AS RNase	No. of perished mice before the killing term	No. of mice with degenerated tumours in day of perishing or killing	Average weight of tumours per 1 mouse in the day of killing (mg)	No. of mice injected with sodium citrate	No. of perished mice before the killing term	No. of mice with degenerated tumours in day of perishing or killing	Average weight of tumours per 1 mouse in the day of killing (mg)
[a]) AS RNase solution mixed with the Crocker cells and after incubation transplanted (killed after 7 days	16	3	16[a]	0[a]	16	2	2	301 ± 68
[b]) As in (a) but AS RNase solution replaced after the incubation by sodium citrate (killed after 14 days)	20	2	18[a]	77 ± 34[a]	14	1	0	599 ± 175
[c]) AS RNase injected inside tumours (killed after 14 days)	8	2	8[a]	1,510 ± 350[a]	8	0	0	2.926 ± 543
[d]) AS RNase injected s.c. from the day of transplantation to the death (killed after 7 days)	11	3	11[a]	57 ± 23[a]	10	0	0	286 ± 64
[e]) As in (d) (killed after 14 days)	9	0	9[a]	50 ± 39[a]	8	0	0	892 ± 372
[f]) As in (d) (killed after 21 days)	47	30[a]	43[a]	0[a,b]	18	4	0	2,266 ± 714
[g]) AS RNase injected s.c. during 8 days beginning the 7th day after transplantation of tumour cells (killed 21 st day	10	10[a]	10[a]	—	10	1	0	2,506 ± 994

[a] Statistically highly significant $P < 0.01$. [b] In 13 mice which survived the 21st day after transplantation of Crocker tumour cells.



Microphotograph of growing, undegenerated Crocker tumour in mouse (a) and tumour degenerated by the effect of AS RNase (b). All cells in the degenerated tumour are lysed. × 450.

0.1 ml sodium citrate was injected once inside a Crocker tumour (8 days after transplantation of tumour cells). Mice were killed 14 days after the AS RNase injection, tumours weighed and histologically evaluated. In further groups of mice (d–g) AS RNase was subcutaneously injected at different time intervals after tumour cell transplantation. A daily dose of AS RNase was 0.35 mg (0.05 ml basic solution). The number of injections, as well as the date of killing the experimental and control groups of mice as well as weight and histological investigations of tumours were different in various groups as regards time aspect (Table).

Results and discussion. The results of study of AS RNase effect on the Crocker tumour cells in mice are summarized in the Table. Through the effect of this ribonuclease, highly significant degenerative changes occurred in all the experimental groups. These changes were demonstrated both in number of animals with degenerating tumours and in the weight of tumours. Histological proof of the degenerative processes is documented in the Figure. In groups of mice injected with AS RNase, however, higher mortality occurred when compared with control animals. This is shown especially in the groups (f) and (g) where the term of killing the animals was fixed at the 21st day after tumour cell transplantation. From the total number of 121 mice, against tumours of which AS RNase was used, only 6 animals had undegenerated tumours. In control groups, from 81 mice 73 animals had healthy, undegenerated tumours in planned terms of killing. The mortality of experimental mice was evidently caused by the intoxication with the disintegrating elements of tumour cells, judging by the fact that in all the experimental mice which died tumours had degenerated. The second proof that the mortality is not attributable to direct toxic effects of ribonuclease can be derived from the longtermed injections of this enzyme to 4 tumour-free mice. These mice did not die after 25 doses of AS RNase (0.35 mg daily).

Zusammenfassung. 121 Mäusen mit Crocker Geschwulst wurde Bullensamen-Ribonuklease injiziert. Bei 115 Tieren konnte die Geschwulstdegeneration nachgewiesen werden während bei 81 Kontrolltieren eine Degeneration nur in 8 Fällen eingetreten ist. Es scheint, dass die Ribonukleinsäure der Crocker Geschwulst von Mäusen ein geeignetes Substrat für die enzymatische Aktivität der Bullensamen-Ribonuklease findet..

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Radiation-Induced Leukemia in Rats: Synergistic Effect of Urethan

Previous investigations have shown that rats are relatively resistant to induction of leukemia by X-radiation alone[1–3]. The induction of leukemia in mice, either by X-radiation or other agents, can be greatly accelerated by the concomitant administration of urethan[4,5], a compound which is itself weakly carcinogenic[6]. The possibility that urethan (ethyl carbamate) could be used to promote the induction of leukemia in irradiated rats was therefore investigated.

Methods. Each group of rats consisted of approximately equal numbers of male and female animals of a black hooded 'Collip' strain. The control group contained 50