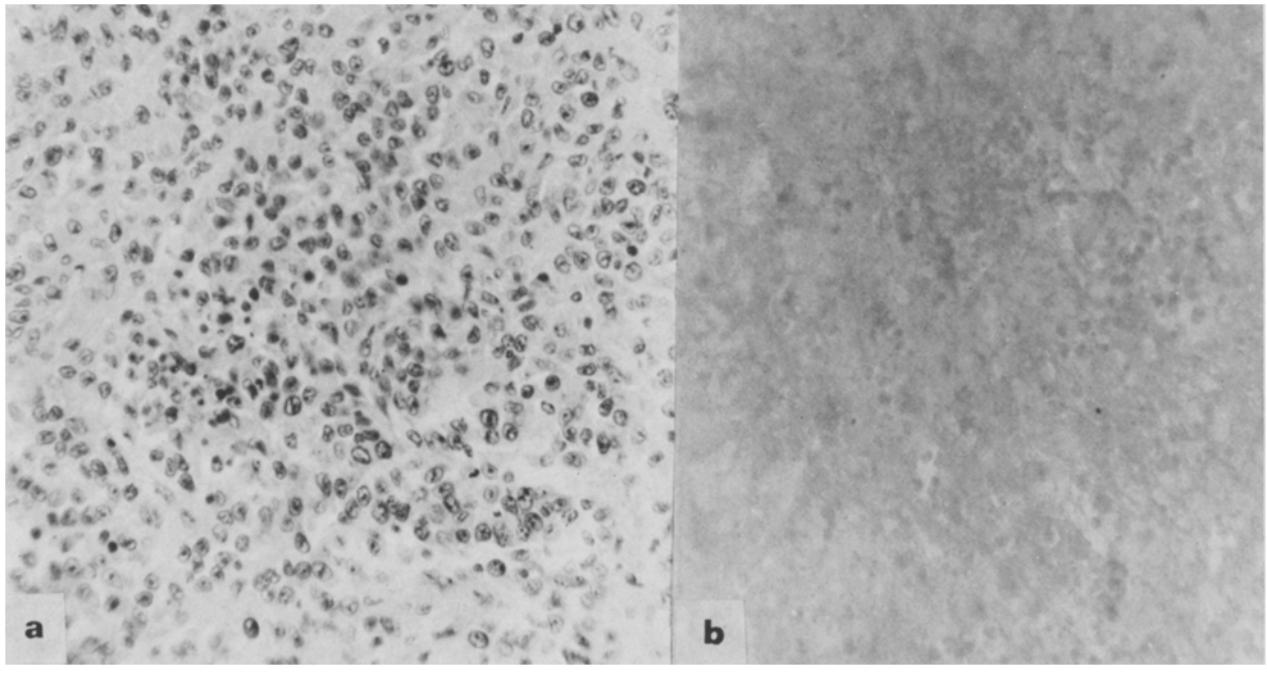

Microphotograph of growing, undegenerated Crocker tumour in mouse (a) and tumour degenerated by the effect of AS RNase (b). All cells in the degenerated tumour are lysed. × 450.

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

Tumor induction in rats with X-radiation and urethan

Group	Treatment		Age at first treatment (weeks)	Animals with tumors (%)		
	X-ray dose (R)	Urethan injections		Mammary plus skin tumors	Leukemia	Other tumors
Control	—	—	—	4	2	0
A	—	5×	10	0	8	4
B	55 R	5×	10	7	11	4
C	165 R	5×	10	4	13	4
D	500 R	5×	10	63	33	4
E	5×165 R	5×	10	12	64	12
F	5×165 R	—	10	58[a]	25[b]	0
G	5×165 R	—	0	28	38	9

[a] χ^2 value for F vs. E = 7.2, $p < 0.01$; F vs. G = 4.0, $p < 0.05$. [b] χ^2 value for F vs. E = 4.9, $p < 0.05$; F vs. G = 0.5, $p > 0.3$.

animals while each experimental group contained 23 to 32 animals.

Experimental treatments were as follows: A) 5 i.p. injections of 0.9 g urethan/kg at intervals of 1 week; B–D) 1 initial exposure of X-rays (300 Kvp) to the whole body followed by 5 weekly injections of urethan as above; E) 5 weekly exposures of 165 R X-rays plus 5 weekly injections of urethan, each injection being given a few min after X-irradiation; F) 5 weekly exposures of 165 R X-rays without urethan. All the above treatments commenced when the rats were 10 weeks of age. Since newborn mice are often more susceptible to induction of leukemia than are adult mice, another group of rats (G) was exposed to 5 weekly doses of 165 R X-rays with the first dose being given approximately 3 h after birth. The surviving animals were sacrificed for autopsy and examination of the blood picture either when they became ill and appeared moribund, or at 70 weeks of age.

For comparison, another group of 5-week-old rats was injected i.v. 5 times at 2 week intervals with 35 mg 7, 12-dimethylbenz(α)anthracene (DMBA)/kg[7,8]. DMBA in lipid emulsion was kindly supplied by P.E. SCHURR, The Upjohn Company, Kalamazoo, Michigan. All of the animals in this group were dead within 24 weeks after the first injection, 82% of them having been diagnosed as leukemic at the time of death.

Results. The results are summarised in the Table. Mammary (female rats) and skin (male rats) tumors were grouped together since both appeared to be sex-limited and hormone-dependent. The skin tumors, of which there were on occasion up to 10 per rat, were slow-growing, keratinised tumors derived from the hair follicles and sebaceous glands[9]. The incidence of mammary and skin tumors, in contrast to that of leukemia, was diminished by urethan treatments (groups E and F) and by irradiation at an early age (groups F and G).

In all, 50 cases of leukemia were diagnosed in the 180 animals treated with urethan and/or irradiation. 30 of these cases appeared to be uncomplicated leukemias similar to those found after DMBA injection. Livers and spleens were frequently enlarged up to 15 and 2.4%, respectively, of body weight, as compared with < 4 and < 0.45% in our control animals. 9 other cases were associated with malignant lymphomas, 6 were associated with other solid tumors in the same animal, 3 involved widespread lymph-node proliferation in the peritoneum, while 2 were associated with thymoma. The average time to death after the first treatment was 49 weeks for all leukemias in groups A–F or 39 weeks for those in group E alone, while the average time to death in the DMBA-injected animals was 15 weeks.

These leukemias could be successfully transferred into adult rats which had been exposed to a sub-lethal dose (500 R) of X-radiation to depress immune responses. Out of 13 animals injected i.p. with 2 ml heparinised blood from leukemic donors, 12 developed a leukemia which proved fatal within 3 to 11 weeks. Injections of liver or spleen homogenate were also successful in transferring into irradiated recipients.

Further experiments are required to establish reliable dose-effect relationships. However, these preliminary results indicate that radiation-induced leukemia can be promoted in rats as well as in mice by administration of urethan after irradiation (Table). This observation is of interest in view of current discussions on possible differences in the mechanism of induction of leukemia in mice and in rats[3,8,10].

In summary, the leukemogenic effects of urethan and X-radiation together (group E) were greater than the sum of their separate effect (groups A and F). However, the observed number of mammary and skin tumors induced

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by the combined treatment was less than after X-radiation alone.

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Zusammenfassung. Nachweis, dass i.p.-Injektion von Urethan und Ganzkörper-Röntgenbestrahlungen bei Ratten eine potenzierte leukämogene Wirkung hat, während die Zahl der entstandenen Haut- und Mamma-Tumoren geringer war als bei der Einzelbehandlung.

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Survival and Endogenous Colony Formation in Irradiated Mice Grafted with Normal or Infectious Mononucleosis Bone Marrow

Previously we attempted[1,2] to induce growth of a human haemopoietic cell line in lethally irradiated mice. Since cytotoxic drugs were found to be unable to prevent graft versus host reaction[3], an antihuman antilymphocytic serum was given after the transfer of human bone marrow. In most of the recipient animals, haemopoietic spleen colonies were found. Chromosomal analysis of these spleen nodules revealed a mouse karyotype suggesting an enhanced endogenous stemcell proliferation.

The present investigations deal with the influence of two irradiation doses and try to evaluate the nature of the colony inducing factor in human bone marrow. An increased activity was noted in the bone marrow derived from three infectious mononucleosis patients.

Adult NMRI (Naval Medical Research Institute) mice were used and received a total body irradiation of 850 or 975 rad as described previously[1]. Human bone marrow or buffy coat cells werve given within 3 h after the irradiation. The surviving animals were killed at day 9 and the spleen nodules counted under a dissecting microscope. In some experiments the grafted recipient animals also received 0.10 ml of a horse derived antihuman antilymphocytic serum (ALS), lymphotoxicity titer 1/3000–1/8000.

Results. Heavily irradiated mice (975 r) grafted with human bone marrow showed an improved 9 day survival. This was further enhanced by an antihuman ALS given after the cell transfer (Table I). The highest survival was observed in mice grafted with infectious mononucleosis bone marrow, while none of the animals grafted with cells from different malignant blood diseases (acute leukemia – lymphosarcoma) survived 9 days. Most of the irradiated and ALS treated animals grafted with human bone marrow showed haemopoietic colonies at the spleen surface. ALS alone did not induce colony formation although it slightly increased the survival. Chromosomal analysis of these colonies revealed a pattern of 40 acrocentric chromosomes, typical for a mouse karyotype (Figure).

In contrast to the 975 r irradiated mice, grafting of human cells into 850 r irradiated animals markedly decreased the survival (Table II). When the grafted mice were also treated with ALS, the survival was equal to the irradiated ungrafted recipients, Grafting of infectious mononucleosis bone marrow resulted in a survival similar to the normal human bone marrow and ALS-treated mice. Like in the 975 r, grafting of human cells and ALS treatment resulted in haemopoietic spleen colony formation.

Peripheral blood buffy coat cells derived from infectious mononucleosis patients given to the 850 r irradiated and ALS treated mice gave a doubling of the 9 day survival, compared to normal buffy coat cells (32% versus 16.6%). In both groups spleen colonies were found.

As a control, the influence of different samples of cell-free human plasma (normal – infectious mononucleosis) was tested. Only a small increase in survival and a few colonies were observed. There was a slightly higher survival in the animals treated with normal plasma compared to the mice who received plasma from a infectious mononucleosis patient.

In a last experiment, the colony-inducing ability of in vitro irradiated human bone marrow (10,000 r) injected into 975 r and 850 r irradiated mice was tested. The

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Table I. Endogenous colony formation in 975 r irradiated anti-human ALS treated and /or human bone marrow grafted mice

Grafted bone marrow	ALS	9 day survival %	Animals with splen colonies (%)	Mean number of colonies positive spleen		
				Mean	S.D.	Range
Nihil	–	3.33 (1/30)	0	–	–	–
Nihil	+	13.3 (2/15)	0	–	–	–
Normal	–	11.7 (4/34)	50 (2/4)	1	–	1.1[b]
Normal	+	25 (14/54)	78.5 (11/14)	3.2[a]	2.97	1–11
Infect mononucleosis	+	61.5 (8/13)	75 (6/8)	2.8[a]	3.03	1–8
Malignant blood disease	+	0 (0/90)	–	–	–	–

( ); number of animals. [a] One recipient mice, having spleen colonies too numerous to be counted, was not included in the calculation of the means. [b] Individual number.