Reports

Implantation of Normal Blood-Forming Tissue in Radiated Genetically Anemic Hosts

Moderate x-radiation (200 r, whole body) affects a particular type (WW^v) of genetically anemic mice (*1*) more than it does their normal (*ww, Ww*) littermates. The radiation reactions of littermate pairs of adult mice, isogenic except for their *W*-genotype, were compared, using animals from two different interstrain F_1 hybrid crosses (*2*). These two groups consisted of offspring from matings of *Ww* parents from strains C and K with W^vw parents from a stock maintained isogenic with C57BL/6 by backcrossing in each generation (now b.c. 30 to 32). Strains C and K are strains favoring longevity of *WW* lethals; they were developed by selection and inbreeding [now F_{21} and F_{17} (*3*)]. As adults, anemic WW^v mice from each of these crosses have an erythrocyte count of 6 to 7×10^6 RBC/mm^3 and a hematocrit mean of approximately 39 percent, while normal littermates show an erythrocyte level of 11 to 12×10^6 RBC/mm^3 and a hematocrit mean near 49 percent. The mean and range of erythrocyte volume is 41.0 mμ^3 (36 to 44) for normals, 57.6 mμ^3 (51 to 66) for anemic mice.

Ten normal mice (5 C, 5 K, Table 1, controls) showed below normal hematocrits 5 and 9 days after radiation but had regained essentially normal levels by the 18th day. Nine anemic mice (4 C, 5 K, Table 1, controls) showed continued reduction in hematocrit level at 5, 9, and 18 days after radiation. Greater depression was observed in anemics of the K group than in those of the C group. Between 9 and 18 days, three of five K-anemics and one of four C-anemics died. The surviving anemics in both groups approached normal anemic hematocrit level by the 27th or 45th day. Determinations of mean cell volume 45 to 160 days after radiation indicated that normals and anemics were each forming cells characteristic of their genotype.

Nine normals (5 C, 4 K) and 8 anemics (4 C, 4 K) (Table 1, isologous injected) were given the same treatment as the control group, followed within 1 hour by tail-vein injection of approximately 8×10^6 cells from hematopoietic liver of 15.5-day-old C57BL/6 (*ww*) embryos. In normals of this group, the hematocrit drop was less than in controls, a fact suggesting almost immediate functional or mitotic activity of these injected cells. In anemics, this activity was even more apparent. The differences in hematocrit mean between injected and control anemics at 5 and 9 days represent for the total erythron approximately 3×10^9 and 6×10^9 erythrocytes in the K group, and 2×10^9 and 9×10^9 in the C group. This is at least 10 times the number that would have resulted from these embryo hematopoietic cells in normal position in neonatal mice.

From 18 days onward another significant phenomenon appeared. Hematocrit levels and, where established, mean cell volumes indicated that the isologous injected anemics of both groups were forming cells characteristic of normals. This constitutes strong evidence that injected hematopoietic cells from normal embryos became implanted in the radiated WW^v anemic host, where they functioned autonomously according to their own (*ww*) genotype. The slightly below normal hematocrit levels and somewhat larger than normal mean cell volumes in these anemics are most probably the result of the continued proliferation of WW^v cells but could conceivably indicate an influence of the host on the *ww* cells.

Homologous cells from the liver of 15.5-day-old C3H embryos had no protective effect when they were injected in three irradiated K-WW^v hosts (Table 1, homologous injected). From 5 to 18 days, C-WW^v irradiated anemics injected with C3H cells showed hematocrit levels above those of the controls, but at 27 days there was no evidence of phenotypic alteration. These limited data suggest that under the conditions of this experiment only isologous implanted cells persist for prolonged periods. Special radiation sensitivity of the blood-forming system of the anemic host, apparent in the data on radiated controls, combined with normal antibody-producing capacity as revealed by ability to reject homografts, may make it possible for the first time to separate two aspects of the problem of protection against effects of radiation by implantation of hematopoietic tissue. In former cases, heavier doses of radiation have broken down both blood-forming and antibody-producing systems of the host, so that homologous and even heterologous cells can implant (*4*). It may be significant that in our experiment irradiated anemics implanted with isologous hematopoietic cells are maintaining stable, relatively high hematocrit levels after 160 days.

This functional evidence of bone-marrow chimaera formation is based on ge-

Table 1. Effects on normal and anemic mice of 200-r irradiation, with and without injection of cells from embryo hematopoietic liver. Controls, no injection; isologous injected, injected with 8×10^6 C57BL cells; homologous injected, injected with 8×10^6 C3H cells. Abbreviations and units: Ht., mean hematocrit percentage; MCV, mean cell volume (mμ^3).

Time (day)	K × C57BL F_1 hybrid						C × C57BL F_1 hybrid					
	Control		Isologous injected		Homologous injected		Control		Isologous injected		Homologous injected	
	Ht.	MCV	Ht.	MCV	Ht.	MCV	Ht.	MCV	Ht.	MCV	Ht.	MCV
	Normal (ww, Ww)						*Normal (ww, Ww)*					
0	50.4		48.8		51.3	39.1	47.4	41.6	46.4	42.8	49.0	42.4
5	39.5		45.3		43.8		39.4		42.7		43.5	
9	42.8		45.1		44.7		42.2		44.7		44.1	
18	48.6		49.0		51.0		46.6		48.8		47.6	
27	48.4		50.8		49.1		46.2		48.2		46.2	
45	49.9	41.2	50.2	44.2	49.7	43.7	47.4	42.0	47.0	40.4		
75	49.0	42.7	49.0	42.3	50.2	41.4	46.4	41.5	48.1	41.8		
105	50.1	40.1	45.9	40.9	47.8	39.5	46.6	41.6	47.7	42.5		
160	49.2	39.9	50.5	41.4								
	Anemic (WW^v)						*Anemic (WW^v)*					
0	38.4		40.3		38.7	60.6	39.8	56.8	38.0	58.3	40.2	60.5
5	18.0		22.0		18.2		24.9		27.7		29.2	
9	10.6		18.2		13.9		14.6		26.1		16.8	
18	7.9		37.5		7.9		10.8		44.6		21.6	
	(3d) –				(2d) –		(1d)					
27	23.2		46.1		22.7		38.1		46.3		34.3	
45	33.5	64.9	48.1	44.2	35.7	59.6	30.4	58.8	44.9	44.1		
75	34.4	58.6	46.8 (1d)	42.6	35.5	61.1	30.7	54.3	44.3	46.2		
105	38.3	61.3	45.8	43.6	37.8	67.6	35.0	58.2	44.3	46.4		
160	36.3	57.5	46.7	43.7								

netically controlled autonomy in rate and type of hematopoiesis of normal *ww* embryo cells implanted into irradiated WW^v anemic hosts. The concept of cell implantation is also supported by other data, including differential behavior of isologous and homologous implants. These data corroborate completely the recent evidence (*4*) that blood-forming tissue can implant successfully in irradiated host animals. They further give evidence that, insofar as *W*-gene effects are concerned, such implanted tissue acts in blood-formation according to its original genotype rather than in conformation to correlative influences from the body of the host.

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References and Notes

1. E. S. Russell, in *Aspects of Synthesis and Order in Growth*, D. Rudnick, Ed. (Princeton Univ. Press, Princeton, N.J., 1954), pp. 113–126.
2. This work has been supported in part by a grant to the Jackson Memorial Laboratory from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and by contract AEC-1800 with the U.S. Atomic Energy Commission.
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Preparation of Nucleic Acids from Ehrlich Ascites Tumor Cells

Biological studies in progress at this laboratory have made it desirable to obtain undegraded nucleic acids from Ehrlich ascites tumor cells. A previous paper (*1*) described the physical properties of deoxyribonucleic acid (DNA) isolated from the tumor cells by several different methods.

This is a preliminary report on the isolation of highly polymerized ribonucleic acid (RNA) from Ehrlich ascites tumor cells and a comparison of nucleic acids prepared directly from the cytoplasm or nuclei with those isolated from the nucleoproteins.

Deoxyribonucleic acid prepared directly from Ehrlich cell nuclei by the method of Kay, Simmons, and Dounce (*2*) is highly polymerized and compares favorably with other material reported in the literature (*2*). Isolation of DNA protein by the method of Mirsky and Pollister (*3*) using 1*M* NaCl followed by duponol extraction also yields highly polymerized material. In contrast, DNA extracted in the same manner from DNA protein prepared by the distilled water method of Stern and others (*4*), and Gadjusek (*5*) is a degraded polydisperse material with an intrinsic viscosity of 5.2 in 0.2*M* NaCl.

A similar observation was made with RNA preparations. Ribonucleic acid, extracted by the method described in a subsequent paragraph from the ribonucleoprotein fraction obtained by the method of Mirsky and Pollister (*3*), is polydisperse and of low molecular weight. That derived directly from a cytoplasmic extract is paucidisperse and contains highly polymerized material. It is doubtful that the physical-chemical methods used in isolating the nucleoproteins are responsible for the depolymerization of the nucleic acids. Enzymatic degradation is a more reasonable explanation and has been discussed by Chargaff (*6*) and by Magasanik (*7*). In the case of Ehrlich ascites tumor cells, enzymatic degradation may be a more important factor in the isolation of RNA than of DNA, for the cells appear to be almost completely devoid of DNAse activity. Attempts to isolate highly polymerized RNA from Ehrlich cells employing duponal (*8*) or guanidine hydrochloride (*9*) have failed.

By the following procedure, RNA of high molecular weight was obtained. Ascites cells were frozen rapidly in a mortar immersed in Dry Ice-alcohol, then ground to a fine powder. The powder was homogenized in 0.14*M* NaCl containing 0.01*M* sodium citrate adjusted to *p*H 7. The nuclei were removed by centrifugation at 3000 rev/min for 20 minutes, and the supernatant was filtered. Ribonucleic acid was derived from this cytoplasmic fraction by extraction with phenol by the method of Gierer and Schramm (*10*). The RNA thus obtained was highly reproducible. Identical preparations were made from several batches of cells, both those that had been freshly harvested and those that had been held for several weeks at a temperature of −70°C. Yields were of the order of 1.0 to 1.5 g of RNA per 100 g wet weight of cells.

The RNA had a N/P ratio of 1.6 to 1.7. All the phosphorus could be accounted for as ribonucleotide phosphorus. The biuret reaction for protein was negative with samples as large as 40 mg, indicating that if protein is present, its concentration must be less than 0.5 percent. In addition, acid hydrolysis and partition paper chromatography for amino acids failed to show detectable contamination by protein. In 0.14*M* NaCl buffered with 0.01*M* citrate at *p*H 7.0, the material traveled as one peak in the electrophoresis apparatus. In the ultracentrifuge, there were four fast-moving peaks, and about 25 percent of the preparation, of low molecular weight, did not move away from the meniscus.

Table 1. Apparent relative concentration of the several components observed in the ultracentrifuge.

$S_{20°H_2O}$	Relative concentration (%)
16	30
18	10
21	10
24	50

The RNA could be precipitated with ethanol. Drying with acetone appeared to cause partial denaturation and loss of solubility. The material was soluble in distilled water. The components of high molecular weight were slowly but more or less completely precipitated if the solution was made 1*M* with respect to NaCl. The material of low molecular weight remained in the supernatant. The RNA was stable for at least 72 hours at 4°C. The measured sedimentation constants dropped markedly in 10 days.

A sample of high-molecular-weight RNA was prepared for physical studies by precipitation with 1*M* NaCl. Its concentration was estimated by nitrogen analysis, assuming that RNA contains 14 percent nitrogen. Table 1 lists the apparent relative concentration of the several components observed in the ultracentrifuge when an RNA solution of 0.4-percent concentration was employed. The dependence of the sedimentation constants on concentration was small, and the measured intrinsic viscosity was 0.35. The ultraviolet absorption spectrum is shown in Fig. 1. The absorption maximum was at 257 mμ with $E_1{}^{1\%}{}_{cm}$ = 210.

The high-molecular-weight RNA appears to be a compact molecule and is quite different from DNA. One is

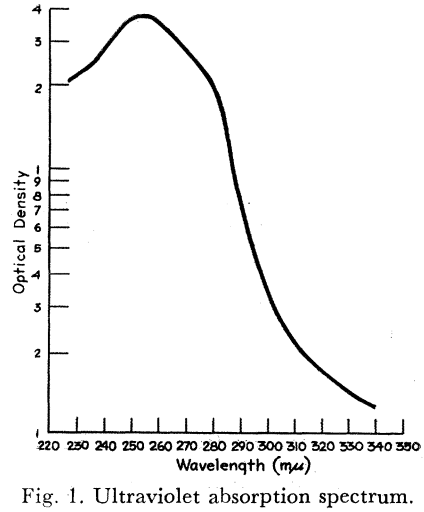

Fig. 1. Ultraviolet absorption spectrum.