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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27 August 1956

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

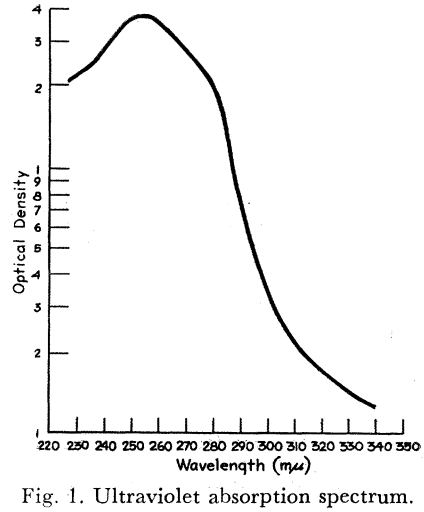

Fig. 1. Ultraviolet absorption spectrum.

tempted to speculate that the chemical structure of highly polymerized RNA is perhaps different from that of DNA and contains cross links at the 2-position of the ribose. A purified sample of the $S = 24$ component should be very useful for fundamental studies of the structure of RNA. Attempts are being made to obtain homogeneous material (*11*).

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Age, Growth, and the LD_{50} of X-rays

In the case of x-rays and other ionizing radiations, the dependence of the acute LD_{50} on age has not been determined throughout the life-span of a mammal, although it is known that immature mice (*1*) and rats (*2*) are more sensitive than mature ones, and it has been indicated that mature female rats are more sensitive at 16 months than at 6 months (*3*). The present experiments contribute data for the first 75 to 80 percent of the ~900-day life-span of CAF_1 mice.

The biological, physical, and statistical methods employed are described elsewhere (*4*). The mice were weaned at about 30 days and experienced puberty at about 50 days. The radiation factors were 250-kvcp x-rays; half-value layer, 1.6 mm Cu; tissue-dose rate, ~35 r/min. Deaths were counted during the 28-day period after a single whole-body exposure. For each LD_{50} determination, there were 4 to 6 dose-groups of about 10 animals each, thus employing a total of about 50 animals.

The results (Fig. 1) showed that the LD_{50} was a linear function of log age, A, from 37 to 105 days (*5*) but was practically constant from 115 to 709 days (*6*). Thus the hypothesis that the "adult" LD_{50} is proportional to remaining life-expectancy (*7*) was not confirmed.

A change occurred in the response of the oldest animals, shown by the increased value of S, the average of the LD_{84}/LD_{50} and LD_{50}/LD_{16} ratios. For ages 600 to 709 days, three determinations of S were 1.16, 1.17, and 1.24. For ages 37 to 434 days, the mean and standard deviation of 29 determinations of S were 1.072 ± 0.024. Radiobiologically, therefore, the population became more variable at 600 days.

The diphasic dependence of the LD_{50} on log age (Fig. 1) was similar to that for log body weight (*8*), a parameter of growth. It may be said, therefore, that for the first 80 percent of the life-span the LD_{50} continued to increase until the growth rate fell to its fully mature level, after which it remained constant.

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5. $LD_{50} = -26 + 336\ A \pm 20$; $\hat{\sigma}$ (336) = 35.
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8. Let W = log weight (g). For males, W(37–103 days) = $0.43 + 0.50\ A \pm 0.02$; W(115–709 days) = $1.30 + 0.083\ A \pm 0.02$. For females, W(37–103 days) = $0.54 + 0.41\ A \pm 0.02$; W(115–600 days) = $1.08 + 0.14\ A \pm 0.02$.

27 September 1956

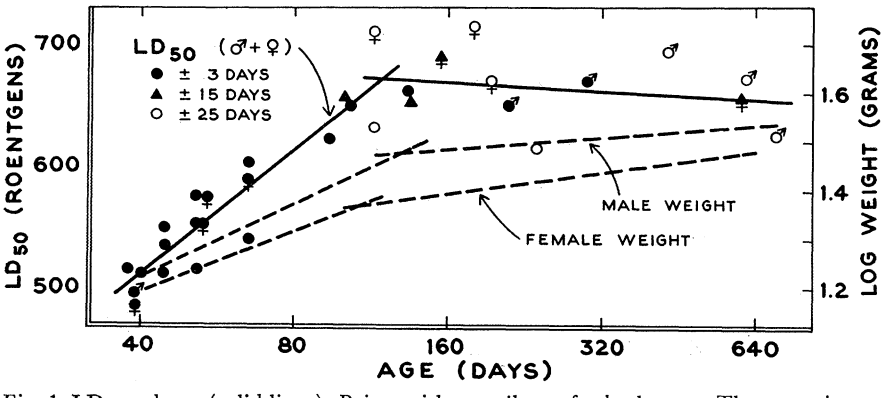

Fig. 1. LD_{50} and age (solid lines). Points without tails are for both sexes. The range in age for each point is indicated by the symbol. The dashed lines relate log body weight to age for the animals of these experiments.

Fowl Pox Vaccine Associated with Parthenogenesis in Chicken and Turkey Eggs

Studies were initiated at the Agricultural Research Center, Beltsville, Maryland, in 1953 to determine which factor or factors were responsible for the parthenogenetic development encountered in the eggs of chickens (*1*) and turkeys (*2*). Since these studies were initiated more than 13,000 infertile chicken eggs have been incubated and examined for parthenogenesis. These eggs were produced by chickens of four different breeds—namely; New Hampshires, Barred Rocks, Rhode Island Reds, and Dark Cornish—as well as two crosses involving New Hampshires and Dark Cornish. Data obtained showed an inherited tendency on the part of certain individuals, as well as on the part of different breeds, to produce eggs that will develop parthenogenetically. The eggs of Dark Cornish and Cornish crosses were the only ones encountered showing any appreciable amount of parthenogenesis that could be detected macroscopically. The development in the case of chicken eggs consisted typically of a limited growth of membranes. These data also indicate that this tendency can be increased or decreased by selective breeding. The causal agent remains unknown. However, it was found in 1955 that a virus may be involved in the initiation of parthenogenetic development in the eggs of Dark Cornish chickens.

In 1955, the eggs laid by 42 pullets from 19 January to 4 May were incubated and examined for parthenogenesis. On 5 May, these same 42 pullets were vaccinated, 13 with pigeon pox and 29 with fowl pox vaccine. All eggs laid by these 42 females during the following 4 months were likewise examined for parthenogenesis. In each case the method of testing was the same, an initial 9- to 10-day period of incubation, following which the eggs were broken and the germinal discs were examined macroscopically for parthenogenetic development.

In 1956, the eggs of 35 Dark Cornish females were examined before and fol-