Christmas Factor: Dosage Compensation and the Production of Blood Coagulation Factor IX

Abstract. *The amount of factor IX (Christmas factor) for different genotypic classes was determined by means of a variant of the thromboplastin generation test. The mean value for females heterozygous for the Christmas gene was about half the mean values for normal males and for normal homozygous females; means for the latter two groups were about equal. This dosage compensation is interpreted as evidence in support of Lyon's hypothesis, according to which one X chromosome is inactive in mammalian females.*

On the basis of evidence from mouse genetics, Lyon (*1*, *2*) proposed the hypothesis that in each cell of a mammalian female one of the X chromosomes is genetically inactive. Beutler, Yeh, and Fairbanks (*3*) independently arrived at the same conclusion from a study of women heterozygous for the gene controlling glucose-6-dehydrogenase deficiency. The finding that intrapair differences are more often greater in monozygotic female twins than in monozygotic male twins was interpreted as evidence in support of Lyon's hypothesis (*4*). Cytological data for both the mouse (*5*) and man (*6*) also support the hypothesis by showing that in females one X chromosome behaves differently from the other in being heteropycnotic.

Lyon's theory has an important implication which may serve as a basis for testing it. If the theory is correct, no gene in the X chromosome should fail to show dosage compensation (that is, equal expression in normal males and females), since normal homozygous females are supposed to have active genes in a single dose, like hemizygous males. In heterozygous females, the expression of the normal trait is reduced if the gene is semidominant, because heterozygous females must have an active normal allele in only part of their cells. If the mutant allele is an amorph, in heterozygotes the expression of the trait must be reduced, on the average, to half (Table 1). Therefore, the finding in mammals of any sex-linked gene which does not show dosage compensation would be strong evidence against Lyon's theory.

We have tested this implication for the case of the Christmas disease gene. The fact that the mean amount of factor IX (the Christmas factor) is smaller in carrier than in noncarrier females has been reported by several authors (*7*). However, a comparison of the amounts and distribution of factor IX in normal males and in normal homozygous females was crucial for the point we are discussing. Therefore, using a variant of the thromboplastin generation test, we determined the amounts of factor IX in pooled samples from three groups: (i) 100 normal women considered homozygous, since they had negative personal and family histories; (ii) 17 women (from 11 different families) known from pedigree evidence to be heterozygous for the normal allele of the Christmas factor gene (only those with at least one son and one direct male ancestor affected were included); and (iii) 100 normal men.

The thromboplastin generation test, as described by Biggs and Douglas (*8*) was adapted for use as an assay for factor IX. The following reagents were used:

1) Saline extract of platelets obtained from pooled normal plasma.

2) $Al(OH)_3$—treated plasma prepared from blood samples of 50 normal individuals, diluted with saline (1 : 5).

3) Serum diluted with glyoxaline buffer, *p*H 7.37 (1:20) and further mixed (1 : 4) with Christmas disease serum. The Christmas disease serum was a dilution (1:10) of a preparation obtained by adding 1.0 ml of calcium chloride (0.025*M*) to 0.1 ml of brain extract diluted (1 : 100) in 1.0 ml of citrated plasma.

4) A pool of platelet-poor normal plasma (as substrate).

The reagents were stored in small amounts for one day's work at − 25°C and used during a single month. The test and control sera were tested on the day after withdrawal of the blood samples.

On the day of testing a standard serum (concentration of factor IX, 100 percent) was prepared from a pool of 80 normal sera which had been collected previously and stored in small aliquots at −25°C. Samples containing factor IX in concentrations of 50, 25, and 12.5 percent were obtained by mixing 1 part of the standard serum with 1, 3, and 7 parts, respectively, of the diluted Christmas disease serum. These samples were assayed by the thromboplastin generation test and a log-log plot of the minimum clotting times as a function of concentration yielded a straight line. The test sera were assayed by the thromboplastin generation test, and the minimum clotting times were read as the factor IX concentration in the log-log graph.

All determinations were made with blind testing procedures and uniform technique. The control for the determinations for groups i and ii was a determination from pooled blood taken from 80 normal females with no cases of hemorrhagic disease in their families. The group-iii sample (that for 100 normal men) was divided into two subsamples. One of them was tested against a second pool of blood taken from the 80 normal females; the other, against pooled blood taken from 80 normal males. Since factor IX was found to be homogeneously distributed in the two subsamples ($t^{98} = 0.115$; $P > .90$), the subsamples were pooled for purposes of our study.

The results of our determinations are condensed in Table 1. The mean amount of factor IX in the heterozygous females is about half that in the normal homozygous females. This shows that the gene is semidominant—that is, the expression of the trait is dependent on the gene dose. However, normal males, with one gene dose (hemizygous), and normal homozygous females, with two gene doses, have on the average about the same amount of factor IX ($z = 0.29$; $P > .75$). These results define a situation where a mechanism of dosage compensation must be at work and are perfectly compatible with Lyon's theory.

It is expected from Lyon's theory that the range of the distribution of

Table 1. Distribution of factor IX (in percentages of the amounts in pooled normal blood) in three classes of normal individuals, as compared with the expected means for the dosage-compensation and no-dosage-compensation possibilities.

Classes	Observed				Expected means (%)	
	N	Mean (%)	Range (%)	Coefficient of variation	Dosage compensation	No. dosage compensation
Homozygous females	100	92	30–200	38.5	92	92
Heterozygous females	17	45	10–100	46.7	46	46
Males	100	90	20–200	36.9	92	46

factor IX for heterozygous females may reach the mean level for affected males at the one extreme and that for normal males and homozygous females at the other (*2, 3*). These extreme values correspond to the case where the X chromosome carrying the mutant gene happens to be active, or inactive, in all cells of the individual. In our data (Table 1) the range for heterozygous females is in agreement with this expectation, since in a sample of only 17 women the range was 10 to 100 percent (in four affected males studied, results from 3 to 10 percent were obtained). Also, the coefficient of variation (Table 1) was greater for the heterozygous females than for the other two classes (*9*).

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Variations in Survival Time after Whole-Body Radiation at Two Times of Day

Abstract. *Rats, anesthetized with sodium pentobarbital, given 900 roentgens of x-radiation over the entire body, survived more than 130 days when the radiation was given in the morning. The same dosage killed all the animals within 13 days when the radiation was given at night.*

In the course of experiments designed to study protection against the effects of whole-body radiation, it became necessary to irradiate animals both in the morning and at night. It was noted that untreated, control animals responded quite differently to the same dose of x-radiation administered at two different times of day. The phenomenon was so striking that a more detailed investigation was made. The experimental animals used were female rats (*1*) weighing 172 ± 3 g. All the animals were kept in quarantine in our animal quarters for 2 weeks prior to the tests and routinely checked by culture for parasitic or bacterial infection. Before and after irradiation the animals were maintained one to a cage and fed a standard rat diet. They were allowed to eat and drink all they wanted. The room in which all the animals were kept was artificially illuminated for 9 hours and kept dark for 15 hours each day. There were no windows. The period of illumination began at 7 A.M.; the period of darkness, at 4 P.M.

Table 1. Results for four experiments.

Irradiation		Animals (*N*)	Average survival time (days)	Days after irradiation for 1st death	Days after irradiation for last death
Date (1962)	Time				
11 June	9 A.M.	5	*		
	9 P.M.	5	8.2	5	10
21 June	9 A.M.	5	*		
	9 P.M.	5	9.2	7.5	13
14 August	9 A.M.	5	*		
	9 P.M.	5	11.0	10	12
28 August	9 A.M.	5	*		
	9 P.M.	5	8.0	6	12

* All animals still surviving on 20 October.

The radiation was administered with a Picker Vanguard high-frequency, deep-therapy unit of 280 kilovolt peak, operating at 20 ma, with added filtration of tin (¼ mm), copper (½ mm), and aluminum (1 mm).

The beam had a half-value layer of 2.03 mm of copper. A 50-cm target-to-midline distance and an air dose of 900 roentgens (given at 93 r/min) were used in all cases. The dosage rates were checked at each irradiation with a Victoreen roentgen chamber and meter calibrated by the National Bureau of Standards. The unit was equipped with a dose-rate meter in the useful beam, which measured the constancy of the output.

The animals were anesthetized with sodium pentobarbital given intraperitoneally (30 mg/kg) and examined before and after radiation for signs for cyanosis. They were placed on a rotating table (12 rev/min) in the beam to insure homogeneity of dose to each animal. Table 1 shows the results for four experiments and the dates of irradiation. Of the 20 animals irradiated in the morning, all survive at this writing and continue to appear in good health, whereas none of the animals irradiated at night survived longer than 13 days. No signs of cyanosis as a result of the anesthetic were noted either before or after irradiation, and the animals all revived from anesthesia at about the same time.

Work in this laboratory is being continued to determine the basis of the differences in radiosensitivity that were observed (*2*). We believe that these may be light-dark related and that radiosensitivity may vary as a function of irradiation in the light phase or the dark phase of a 24-hour day. We are also investigating the possibility that sex or species differences are reflected in the results.

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Notes

1. The rats used in this study were obtained from Carworth Farms, New City, New York.
2. This work is supported in part by the U.S. Public Health Service (contract USPHS-CRT 5069).

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Incidence of Gastric Ulcers in Swine

Gastric ulcers have been recognized in man for many years. Sporadic observations made in the past (*1*) have also indicated the occurrence of gastric ulcerations in swine. The ulcers in swine are similar to those found in man, and investigations with pigs may furnish some valuable information applicable to humans. A recent study of 164 hogs slaughtered in Indiana (*2*) showed a 25-percent incidence of esophagogastric ulcers. The condition is