on the "human counter." The K^{40} content of group A, of group B, and of both groups together was determined. Apparently, the positioning of the hams in the counter was not of critical importance, since the total number of gamma rays per second recorded for the individual pairs of hams, after the appropriate correction had been made for background depression (21.99 and 22.29 gamma rays per second), agreed closely with the value obtained when all hams were counted simultaneously (44.3 gamma rays per second).

After the K^{40} values for the intact hams had been obtained, the skin and external fat were removed, and counting data were obtained for groups A and B. Following this, the bone and most of the seam fat were removed, and the remaining lean meat was counted for each group. The amount of crude fat present in the lean was determined (*4*) so that the K^{40} values could be related to the amount of fat-free lean present. The data obtained are presented in Table 1.

The K^{40} activity, in actually measured gamma rays per second, per pound, was obtained by dividing the value obtained for gamma rays per second by the actual weight of the sample in pounds. The K^{40} values were not adjusted by a counting efficiency factor to an absolute basis. These values varied directly with the percentage of fat-free lean present. The correlation coefficient (*5*) obtained for the correlation of these two sets of values is 0.983, indicating that the correlation is significant at the 1-percent level. The values for measured K^{40} gamma rays per second, per pound varied inversely with the percentage of fat present. A correlation coefficient of -0.966 was obtained from these values, indicating a negative correlation which is significant at the 1-percent level. The values for measured K^{40} gamma rays per second, per pound of fat-free lean were derived by dividing the value for gamma rays per second by the weight of fat-free lean in the sample. The average of these values was 1.60, with a relative standard deviation (*5*) of 4.8 percent for the total variability among different samples.

A statistical evaluation was made of the counting error involved in the counting operation (*6*). The relative standard deviation of the counting measurements in tests with pairs of hams in which the samples were counted for 20 minutes and the background was counted for 30 minutes was about 3 percent. When the four hams were counted simultaneously (58 lb of hams), counting of the sample for 13.33 minutes and of the background for 20 minutes gave a K^{40} value with a relative standard deviation for counting error of about 1.9 percent. On this basis, it may be possible to determine the K^{40} content of a 175-pound carcass (with a relative standard deviation for counting error of about 5 percent) by counting the sample for 100 seconds and the background for 200 seconds.

In order to compare the counting efficiency of the "human counter" with that of a crystal detector, the hams of group B were counted individually with an 8- by 4-inch NaI(Tl) scintillator crystal, mounted about 2 in. below the frozen ham, which rested on a piece of plywood 0.25 in. thick. Eight inches of steel shielding surrounded the sample and detector. One ham was counted for 30 minutes; this count was followed by a 30-minute count of the background. The resulting K^{40} peak for this ham was 29.4 net counts per minute, with a relative standard deviation for counting error of about 6.8 percent. The second ham of group B was counted for 60 minutes; this count was followed by a 60-minute count of the background. The resulting counting rate was 34.6 net counts per minute, with a relative standard deviation for counting error of 3.3 percent. A comparison of the counting efficiency revealed that the "human counter" detected about 21 times as many K^{40} gamma rays from hams under the conditions of the comparison made as the crystal detector did. The "human counter" has an additional advantage in that the positioning of the sample is apparently not of critical importance, while, when a crystal detector is used, the position relative to the detector is of great importance.

Table 1. K^{40} values as related to the amount of fat-free lean and fat in samples of fresh ham.

Sample	Measured K^{40} gamma rays per second, per pound	Measured K^{40} gamma rays per second, per pound of fat-free lean	Percentage of fat-free lean	Percentage of fat
		Group A		
Intact hams	0.70	1.50	47.4	40.0
Hams without skin and external fat	1.14	1.64	69.8	16.4
Lean portion	1.30	1.50	86.3	13.7
		Group B		
Intact hams	0.83	1.61	51.7	33.3
Hams without skin and external fat	1.19	1.68	70.6	14.2
Lean portion	1.48	1.68	88.5	11.5
		Groups A and B together		
Intact hams	0.76	1.56	49.4	37.1

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Composition of Cardiolipin

In a recent, extremely interesting communication on the composition of cardiolipin (*1*), Macfarlane and Gray have reported results which, although admittedly inconclusive, throw considerable doubt on the correctness of the presently accepted concept (*2*) that cardiolipin is a glycerylglycerophosphate derivative containing three phosphoric acid molecules linking four glycerol molecules, the latter being also esterified with one oleic and five linoleic acid residues. Indeed, Macfarlane and Gray consider that their results suggest that cardiolipin is "not a single compound, but a mixture of similar compounds varying in fatty acids." In view of this suggestion, I report, believing it to be of some interest, the results of a preliminary study in which cardiolipin was separated into a number of chromatographically distinguishable fractions each of which possessed the biological activity, as measured by the cardiolipin flocculation test (*3*), of the unfractionated cardiolipin but which differed in the fatty acid moiety.

Fifteen commercial preparations of cardiolipin (*4*) and three preparations made in this laboratory from ox-heart

muscle (5) were chromatographed on a mixture of silicic acid and Celite (6) (3:1 by weight). The cardiolipin was adsorbed on the column from benzene, and the column was developed with chloroform containing ethanol (5 percent by volume). The column was extruded, and the zones were made visible by streaking with an alkaline permanganate solution (7). The zones were cut and eluted with ethanol, and the eluate was concentrated to dryness under reduced pressure at room temperature. Approximately 50 percent of the cardiolipin was retained at the top of the column and could not be moved down the column with 5 percent ethanol in chloroform. The top zone was therefore rechromatographed, with 20 percent ethanol (by volume) in chloroform as the developer, and then gave additional zones.

Each of the cardiolipin preparations could be separated into a number of fractions. The exact number of fractions, however, was not the same for each preparation and varied from four fractions found in one case to eight in another. All fractions were biologically active (3). In every case approximately 20 percent of the material placed on the column was not moved from the top of the column under the conditions of development, and hence there is no reason to suppose that additional fractions could not have been obtained.

Three zones, containing approximately 50 percent of the material placed on the column, were found to be present in each of our preparations. These zones were found in approximately the center of the column when the column was developed with (i) four column lengths (8) of chloroform containing 5 percent ethanol (zone 1); (ii) four column lengths of chloroform containing 20 percent ethanol (zone 2); (iii) eight column lengths of chloroform containing 20 percent ethanol (zone 3).

In an attempt to identify the individual acids which might be expected to be present in the cardiolipin of zones 1, 2, and 3, each zone was reduced in ether solution with excess lithium aluminum hydride; after treatment with water in the usual manner, the ether phase was concentrated to dryness, and the resulting oil, which should contain the alcohols corresponding to the acids originally present in the zone, was fractionated by distillation at a pressure of 10 μ.

In each case, only one such alcohol was found in any one zone. The alcohols distilled at the following temperatures: zone 1, 83 to 85°C; zone 2, 118 to 120°C; zone 3, 132 to 135°C. When the three alcohols were mixed together, it was still possible to separate them by fractional distillation. The fractions distilled in the same temperature range.

The undistilled alcohol from each zone was also examined chromatographically. Only one zone was found in each case. A mixture of silicic acid and Celite was used as the adsorbent, and the column was developed with benzene containing 1 percent *t*-butyl alcohol (by volume).

On the basis of carbon and hydrogen analyses, the ultraviolet absorption spectrum, the reaction with maleic anhydride, and the melting point of the alcohol obtained after reduction with hydrogen in the presence of a platinum catalyst (9), the alcohols obtained from zones 1 and 2 were tentatively identified as 1-dodecanol (corresponding to lauric acid) and 9,12,15-octadecatrienol (corresponding to linoleic acid), respectively. The alcohol from zone 3 was not identified. Analysis indicated the presence of more than one hydroxyl group.

The results are such as would be expected if the usual preparations of cardiolipin were mixtures in which the components differed in their fatty acid moiety.

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References and Notes

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Protective Effect of a Colchicine Derivative in Mice Exposed to X-radiation

The profound effect of colchicine upon mitotic activity has been described in abundant detail (1). Results of numerous cytological studies of a combination of colchicine treatment and x-irradiation have also been reported (1, 2). We find no references to effects of colchicine on survival of mammals given whole body irradiation, possibly because adverse rather than protective action is anticipated.

Table 1. Data on survival of mice given an intraperitoneal injection of 1 mg of trimethyl colchicinic acid methyl ether *d*-tartrate 24 hours prior to x-irradiation.

Irradiation (r)	No. of mice per group	Percentage that survived (28 days)	
		Control	Treated
900	40	70	98
950	20	25	75
1100	15	0	80
1100	20	0	45
1100*	16	0	56

* 0.5 mg of the colchicine derivative.

The primary object of the study described in this report was to alter the sensitivity of bone marrow to x-irradiation. The provisional assumption was made that the sensitivity of these cells would be reflected in survival of the animal. In principle this appears to be a vast oversimplification, although in practice the neutralization of other variables may leave the desired association in evidence. A less distant relationship may be one between marrow sensitivity and peripheral leucocyte count (3).

There does not seem to be convincing evidence that cells in colchicine-induced metaphase are more sensitive to radiation than cells in their normal state, and our expectation slightly favored an increase rather than a decrease in resistance. The mere fact of an increase in survival would not, of course, indicate a specific increase in resistance of colchicine-treated cells in preference to any of a number of other processes which might be responsible, but the first step was to determine whether or not survival would be altered by pretreatment with colchicine.

The present brief report is preliminary in nature. The mice used were (BALB/c × DBA/2) F_1 females 12 to 15 weeks old, kept in individual cages. A Van de Graaff generator, operating at 2.5 Mev; 0.6 ma; HVL, 1 cm of lead; TSD, 1 m; and dose rate, 250–300 r/min was used for irradiation (4). [Further details are to be found in (5).] The colchicine derivative used was trimethyl colchicinic acid methyl ether *d*-tartrate (N.C.I. No. 1136), some of the characteristics of which have been described by Leiter and his associates (6). This was given as an intraperitoneal injection of 1 mg per 20 to 25-g mouse in 0.2 ml of saline, 24 hours prior to irradiation.

As shown in Table 1, survival was considerably better in mice that had been given the colchicine derivative than in controls, the maximum difference being