# Relation Between Single-Strand DNA Mass and Sedimentation Distance in Alkaline Sucrose Gradients[+]

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## Summary

Single-strand breaks are introduced into T2 and B. subtilis DNA in dilute solution with gamma rays and the DNA sedimented on alkaline sucrose gradients. Assuming (1) the number of single-strand breaks is linear with dose, and (2) the distance sedimented in alkaline sucrose gradients D is proportional to $M^{\alpha}$ (M is the single-strand DNA mass), the value of $\alpha$ is determined to be 0.40.

## Footnotes

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# INTRODUCTION

Alkaline sucrose gradient sedimentation is widely used to determine the size of single-strand DNA - in the determination of the number of single-strand breaks in native DNA, for example. A relation between the distance sedimented and the single-strand molecular weight has been published by Abelson & Thomas (1966).

While measuring double-strand breaks in DNA caused by ionizing radiation (Levin & Hutchinson, 1972), the number of single-strand breaks after various radiation doses was also investigated by sedimentation in alkaline sucrose gradients. After the data were obtained, it became clear that the linear relation which should hold under the conditions used between the number of single-strand breaks and the dose of ionizing radiation, could give information on the relation between the molecular weight of the single-strand DNA and the distance sedimented. Consider a DNA with an initial distribution in molecular weight. After a known dose of ionizing radiation a new distribution, sedimenting at a lower velocity, is found. Assuming a relation between the molecular weight and the sedimentation distance, molecular weight distributions can be fitted to the initial and final sedimentation profiles, and from these the presumed number of breaks introduced can be calculated. This can be done for DNA given various doses, and the presumed number of breaks plotted against dose.

If the correct relation between M and D is used, the number of breaks will be linear with dose. If the wrong relation is used, linearity will not be found.

This approach has similarities in principle to the method used by Burgi & Hershey (1961; 1963) to determine the law relating the molecular weight of native double-strand DNA to sedimentation distance in neutral

sucrose gradients. They compared the sedimentation of whole viral DNA molecules to halves and quarters prepared by shearing.

The presently accepted relation between single-strand DNA molecular weight and sedimentation distance in alkaline sucrose gradients (Abelson & Thomas, 1966) was obtained from the sedimentation of various viral DNAs having measured masses. Although this method has very real advantages, it does depend on the accuracy of the DNA mass determinations, a subject of some complexity and uncertainties (see Freifelder, 1970). To give one example, consider the mass of T2 (or T4) DNA. The most recent determinations, all done with great care, give masses of $132 \times 10^6$ daltons (T2, Leighton & Rubenstein, 1969), $114 \times 10^6$ (T4, Schmid & Hearst, 1969), $119 \times 10^6$ (T4) and $116 \times 10^6$ (T2) (Lang, 1970) and $106 \times 10^6$ (T4, Dubin et al., 1970). For another example, there seem to be questions concerning the mass of T5 DNA (see the discussion in the appendix of Levin & Hutchinson, 1972).

Thus it seems reasonable to see what result can be obtained by methods which are independent of absolute mass measurements, as in the radiation-breakage method used in this paper.

## MATERIALS AND METHODS

Except for the details of the alkaline sucrose gradient centrifugation, materials and methods are identical to those described in the previous paper (Levin & Hutchinson, 1972).

Briefly, B. subtilis 23 thy⁻ cells were labeled with [$^{14}$C] - thymine and lysed by a very gentle procedure in a dialysis cell. Various cell components were degraded to dialysable fragments by treatment with lysozyme, sodium dodecyl sulfate and Pronase. The [$^{14}$C] - labeled B. subtilis DNA (∿ 0.2 μg/ml) was mixed with [$^{3}$H] - labeled T2 DNA (∿ 0.4 μg/ml) and both irradiated with $^{60}$Co gamma rays in a solution containing 0.8 M NaCl, 10 mM tris (tris-hydroxymethyl-amino-methane), 2 mM EDTA (ethylene-diamine-tetra-acetate), 2 mM histidine (Sigma), pH 8.0.

Alkaline sucrose gradient sedimentation. Linear alkaline sucrose gradients, 5-20% w/v sucrose, contained 0.8 M NaCl, 0.2 M NaOH and 4.5 mM EDTA. DNA samples of volume 0.15 ml were carefully layered on top of 4.9 ml of gradient in cellulose nitrate tubes, by the method previously described. The tubes were gently loaded into a precooled SW 50.1 rotor and held 1 hour at 10°C, allowing alkali to diffuse up into the layer to denature the DNA. The rotor was then spun at 25,100 rev/min at 10°C for 4 hr.

After centrifugation, each gradient was unloaded thru a hole pierced in the bottom of the tube, with each of 34 fractions deposited on glass fiber disks, washed, and counted in a liquid scintillation counter as previously described.

## RESULTS

Typical alkali sedimentation profiles of the $^{14}C$-labeled B. subtilis DNA and $^{3}H$-labeled T2 DNA after various doses of gamma rays are given in Fig. 1. Certain qualitative conclusions can be drawn from the curves obtained.

(1) The unirradiated single-strand B. subtilis DNA is certainly larger than single-strand T2 DNA.

(2) After irradiation, both DNA's are reduced in size, with the mean molecular weight of the distribution decreasing monotonically with increasing radiation dose.

(3) Although the B. subtilis DNA is initially larger than T2 DNA, after 15 kilorads it is clearly smaller than T2 DNA irradiated simultaneously in the same solution (Fig. 1). Thus the sedimentation of B. subtilis DNA is reduced more rapidly by irradiation than that of T2 DNA.

For a more quantitative assessment of the data, two assumptions were made.

(A) It was assumed that the relationship between sedimentation distance D in a gradient and the DNA molecular weight M is given by a relation of the form (Doty, McGill and Rice, 1958)

$$D/D_{T2} = (M/M_{T2})^{\alpha} \qquad (1)$$

where $D_{T2}$ and $M_{T2}$ are the parameters for single-strand T2 DNA, and $\alpha$ is the coefficient applicable to alkaline sucrose gradients.

(B) It was assumed that the introduction of single-strand breaks by ionizing radiation is linear with dose, takes place randomly along the DNA and that the mass distribution in molecular weight $f(M)\,\Delta M$, after the production of r random breaks per mass equal to that of a single strand of

T2 DNA, is given by the Montroll-Simha (1940) function

$$f(M)\ \Delta M = \exp\ (-r\ \frac{M}{M_{T2}})\ (r\ \frac{M}{M_{T2}})\ (2 + r\ \frac{Mo}{M_{T2}}\ -r\ \frac{M}{M_{T2}})\ \frac{\Delta M}{M_o} \qquad (2)$$

Here Mo is the initial size of the single-strand DNA, assumed homogeneous in molecular weight. The assumption of homogeneity is clearly correct for T2 DNA, which has an initial size $Mo = M_{T2}$. For B. subtilis DNA the initial size and molecular weight distribution in solution is unknown; in the actual calculations it was found that the same conclusions were drawn if Mo were assumed equal to 4 $M_{T2}$, 6 $M_{T2}$ or 12 $M_{T2}$.

The sucrose sedimentation patterns give the DNA mass distributions in intervals of $\Delta D$, the sedimentation distance. Using the relation between M and D given by equation (1), equation (2) can be rewritten

$$f(M)\ \Delta M = \exp\ (-r\ \frac{M}{M_{T2}})\ (r\ \frac{M}{M_{T2}})\ (2 + r\ \frac{Mo}{M_{T2}} - r\ \frac{M}{M_{T2}})\ \frac{M\Delta D}{\alpha MoD} \qquad (3)$$

A value of $\alpha$ - e.g., 0.40 - was assumed, and the value of the parameter r (breaks per mass of DNA equal to $M_{T2}$) which makes equation (3) give the best fit to a particular experimental gradient was determined by the procedure described in the caption to Table I. This was repeated for each gradient, including DNA given 0 to 15 kilorads. Since the number of breaks r should be linear in the radiation dose R, the values of r determined above were fitted to the expression

$$r = a_o + a_1 R + a_2 R^2 \qquad (4)$$

and the coefficients $a_o$, $a_1$ and $a_2$, together with the standard deviations of these coefficients, determined by a least squares procedure.

Another value of $\alpha$ was then assumed, and the process repeated. The coefficients thus determined are given in Table I.

If the number of breaks r is linear in dose R, the quadratic coefficient $a_2$ should be zero, within experimental error. If it is assumed that $a_2$ has a finite value if it is greater than roughly three times its standard deviation, then $0.36 < \alpha < 0.42$ from the T2 DNA results, and $0.38 < \alpha < 0.50$ from the data for B. subtilis DNA.

Another indication of the correct value of $\alpha$ is the accuracy with which each experimental gradient can be fitted by the theoretical expression (equation 3). An indication of this "goodness of fit" is the sum of the squares of the differences between the theoretical and experimental distributions, also given in Table I. It is clear that for both DNA's the sum goes through a minimum for $\alpha \simeq 0.40$.

For T2 DNA there are more experimental data, and there appears to be less scatter, than for B. subtilis DNA. Thus the former data should be weighted more than the latter. Taking all factors into consideration, it would appear that from these results the most likely value of $\alpha$ in alkaline sucrose gradients is 0.40, and that it is unlikely to be smaller than 0.36 or larger than 0.45.

The value for $a_1$, when $a_2 \simeq 0$, is the number of breaks per mass $M_{T2}$ per unit dose. From Table I, the value depends on the value of $\alpha$, but it is clear that the number is always about 10% higher for B. subtilis DNA than for T2 DNA. In Fig. 2 are plotted the number of breaks r for $\alpha = 0.40$ for the two DNA's.

## DISCUSSION

### Linearity of Single-Strand Breaks with Radiation Dose

The major conclusion of this paper, the value of the parameter $\alpha$ characterising the sedimentation of a single-strand DNA in alkaline sucrose gradients, depends on the assumption that the number of single-strand breaks is linear with radiation dose. Since there is as yet no direct experimental way of measuring this proportionality, the assumption must be supported on the basis of the rather extensive knowledge of the radiation chemistry of dilute aqueous solutions which has been built up over the past 25 years (Allen, 1961: Draganic, 1971).

Briefly, in dilute aqueous solutions the chemical changes in solutes are caused almost entirely by reactions of free radicals produced from the water by the action of the ionizing radiations. If a particular free radical F is produced with a yield of $G_F$ radicals/100 ev of energy absorbed (the figure usually quoted by radiation chemists) then for a dose rate of dR/dt kilorads/sec the free radical will be produced at a rate of $0.965\ G_F\ (dR/dt) \times 10^{-12}$ M/sec. The radical will react with solutes in solution according to the equation

$$0.965\ G_F\ (dR/dt) \times 10^{-12} = k_{FN}N + \sum_i k_{Fi}C_i \qquad (5)$$

where

N is the concentration of DNA nucleotides in moles/liter - in these experiments 0.6 µg DNA/ml, or $1.7 \times 10^{-6}$ M nucleotides.

$k_{FN}$, $k_{Fi}$ are reaction rate constants of the free radical F with constituent N or i.

$C_i$ is the concentration of the $i^{th}$ solute.

The crucial point is that the fraction of the radicals F reacting with DNA sugars, and therefore the number of breaks per unit dose, will stay

constant if N, $k_{FN}$ and $\Sigma\ k_{Fi}\ C_i$ stay constant.

In the experiments described in this paper, fewer than one nucleotide-nucleotide bond in $10^4$ was broken at the highest dose used (15 kilorads). Although 4-5 bases may be altered by the action of ionizing radiations per single-strand break formed (Thorsett & Hutchinson, 1971), the total radiation-induced change in N is still less than one in $10^3$ or 0.1%. Such small changes in N make it most unlikely there will be sufficient change in the DNA configuration to change $K_{FN}$.

To change $\Sigma\ k_{Fi}\ C_i$ significantly, it is necessary that the total value of the sum be changed. The constituents contributing to the sum include histidine (2mM), EDTA (2mM), and tris (10mM). If one of these molecules is destroyed for every water molecule destroyed by the ionizing radiation (G for loss of water $\sim$ 4, Draganic, 1971), then 15 kilorads could destroy only 0.06 mM, or less than 3% of the histidine, say, in solution. The change in $\Sigma\ k_{Fi}C_i$ will certainly be less than this.

The oxygen concentration usually has an effect on radiochemical effects, but for DNA the effect of $O_2$ concentration in the absence of -SH compounds appears small (Howard-Flanders, 1960; Hutchinson, 1961). In any event, the magnitude of the oxygen effect in air-saturated solutions depends on dose only above 30 kilorads (Allen, 1961; Draganic, 1971), or at greater doses than those used here.

The presence of histidine also appears to suppress the effects of long lived radiation products (peroxides?) which might act slowly during the period between irradiation and sedimentation (Freifelder, 1965).

Thus, under the conditions of this experiment the number of single-strand breaks should be proportional to radiation dose.

DNA Sedimentation in Alkaline Sucrose

When Abelson & Thomas (1966) studied the sedimentation in alkaline sucrose gradients of a number of homogeneous viral DNA's, they could extract information on the form of the relation between the distance sedimented D and the molecular weight M from simple plots. The form of the relation, D proportional to $M^{\alpha}$, was one which has been widely used, both before and after their work.

The method used in this work is less useful for determining the form of the relation. In the irradiation a distribution in molecular weight is produced, and relating any parameter of the distribution, the maximum for example, to a molecular weight always involves assumptions concerning the relation between M and D.

Given the form of the relation between M and D, the method of this paper has considerable potential for an accurate determination of the parameters, $\alpha$ in this case. Furthermore, the method has the advantage of providing a determination of $\alpha$ completely independent of the problems of absolute mass determination for viral DNA (see Freifelder, 1970).

In fact, the value of $\alpha$ determined in this work, $\alpha = 0.40$, is the same value found by Abelson & Thomas (1966).

The Single-Strand Break Rates for Different DNAs

The linear coefficient in Table I for B. subtilis DNA is about 10% higher than that for T2 DNA. The most probable rates of breakage for the two DNA's differ by about 15%, as shown in Fig. 2, when account is taken of the quadratic terms, which are indeed of opposite sign for $\alpha = 0.40$. This difference in rates is in part caused by the fact that the average molecular weight of a nucleotide in T2 DNA is about 8% heavier than that in B. subtilis DNA because of the glucosylation of the former (Rubenstein et al., 1961). Thus for an equal rate of attack on the deoxyribose, the

number of breaks per unit mass for the B. subtilis DNA should be about 8% higher.

The additional sugars on the glucosylated T2 DNA could also react with radiation-produced free radicals, and thus somewhat reduce the attack on T2 DNA deoxyribose. This could readily account for the remainder of the difference between the two DNA's.

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## Caption to Table I

Each alkaline sucrose sedimentation profile (as in Fig. 1) of a DNA which had received a gamma ray dose R was fitted by a theoretical Montroll-Simha expression, equation (3) of the text. A suitable value of $\alpha$ - e.g., 0.40 - was assumed, a value of the number of breaks r estimated, and a computer program used to calculate the sum $\Sigma\ (f^i_{exp} - f^i_{calc})^2$. Here $f^i_{exp}$ is the normalized part of the radioactivity sedimenting in the $i^{th}$ fraction of the gradient, and $f^i_{calc}$ is the calculated amount (suitably normalized) in the same fraction. The sum $\Sigma$ was calculated for other values of r, and the number of breaks for a given distribution defined as that value of r which gave the smallest sum $\Sigma$. For a given $\alpha$, the minimum value of the sum $\Sigma$ was roughly independent of dose. The average value of this minimum $\Sigma$ is given in the table for each assumed $\alpha$.

For T2 DNA, Mo in equation (3) was put equal to $M_{T2}$. For B. subtilis DNA, parallel calculations were carried out with Mo = 4 $M_{T2}$, 6 $M_{T2}$ and 12 $M_{T2}$. For the largest B. subtilis DNA used in these calculations, the sample given 1 kilorad, the number-average molecular weight ($\sim M_{T2}/2$) was much smaller than any possible Mo, and the conclusions drawn were essentially independent of the assumed value of Mo. Values in the table are those calculated for Mo = 6 $M_{T2}$.

For each assumed $\alpha$, the values of the number of breaks r were related to the dose R in kilorads using the expression (see equation 4 in the text)

$$r = a_o + a_1 R + a_2 R^2.$$

The values of the coefficients and their standard deviations were calculated by a least-squares procedure, and are tabulated here.

TABLE 1

| DNA | quantity | α .30 | .34 | .36 | .38 | .40 | .42 | .44 | .46 | .50 |
|---|---|---|---|---|---|---|---|---|---|---|
| T2 (9 gradients) | $a_1$ | | | 1.73 ±0.053 | 1.59 ±0.041 | 1.47 ±0.032 | 1.36 ±0.028 | 1.27 ±0.027 | | |
| | $a_2$ | | | 0.0104 ±.0036 | 0.00259 ±.0028 | -0.00283 ±.0022 | -0.00724 ±0.0019 | -0.00954 ±0.0018 | | |
| | $\Sigma_i(f^i_{exp} - f^i_{calc})^2$ (average value) | | | .00052 | .00024 | .00010 | .00012 | .00022 | | |
| B. subtilis (5 gradients) | $a_1$ | 2.53 ±0.25 | 2.09 ±0.15 | | 1.75 ±0.096 | 1.60 ±0.074 | 1.47 ±0.069 | | 1.26 ±0.056 | 1.10 ±0.051 |
| | $a_2$ | 0.114 ±0.015 | 0.0479 ±0.0090 | | 0.0176 ±0.0058 | 0.0093 ±.0045 | 0.0032 ±0.0041 | | -0.0040 ±0.0034 | -0.0079 ±0.0030 |
| | $\Sigma_i(f^i_{exp} - f^i_{calc})^2$ (average value) | 0.0064 | 0.0024 | | 0.00069 | .00051 | 0.00059 | | 0.0013 | 0.0027 |

## Figure Captions

Fig. 1 Alkaline sucrose gradients, sedimented from left to right in an SW50.1 rotor at 25,100 rev/min for 4 hr at 10°C, for DNA given various doses of gamma rays.

- ● $^{14}$C-labeled B. subtilis DNA.
- x $^{3}$H- labeled T2 DNA.
- ——— o kilorads.
- ····· 1 kilorad .
- - - - 15 kilorads.

Fig. 2 A plot of r, the number of single-strand breaks per mass of DNA equal to the T2 single strand, versus radiation dose in dilute solution. $\alpha = 0.40$.

- ● B. subtilis DNA
- x T2 DNA

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Fig. 1

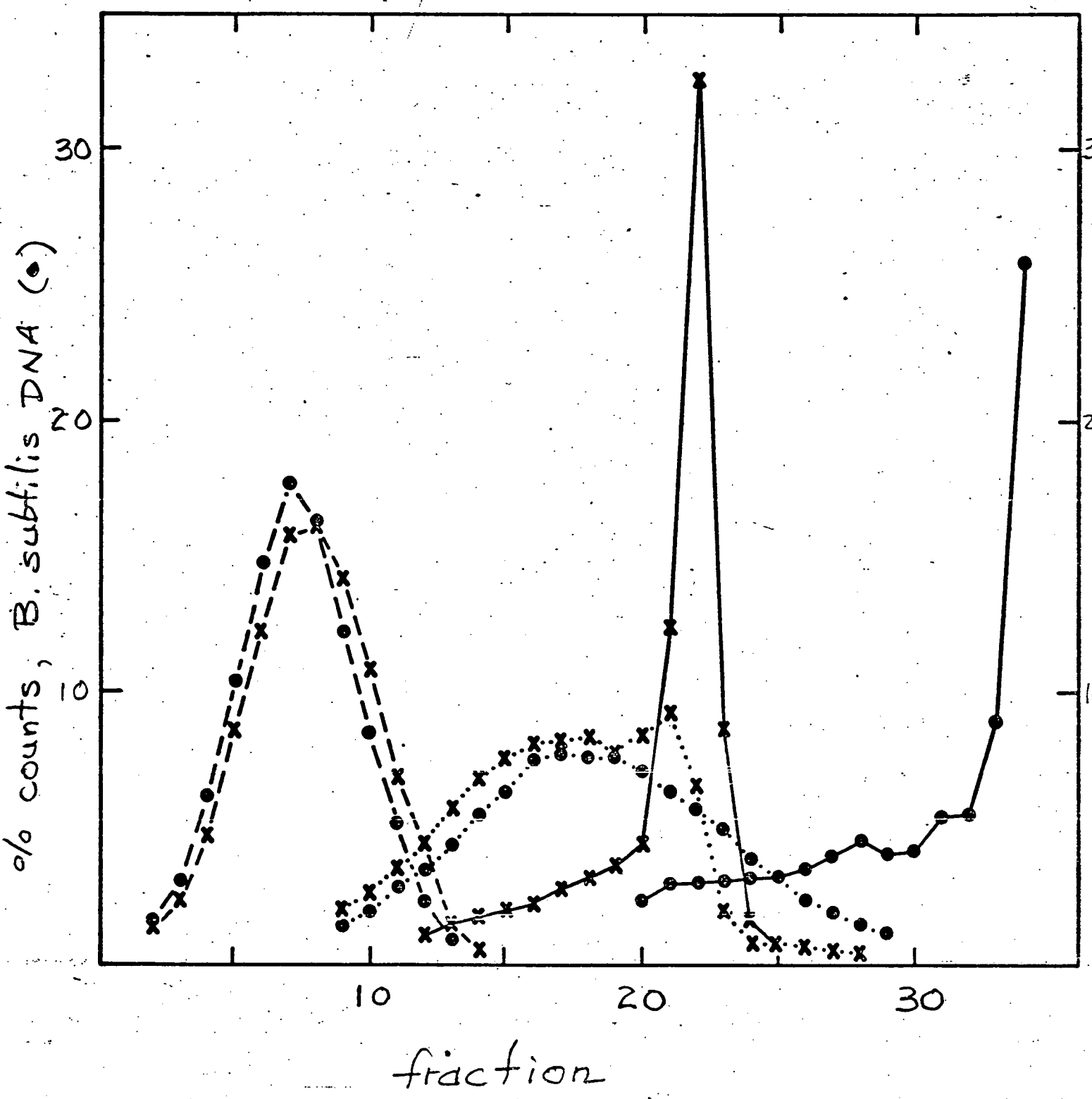

Fig. 2

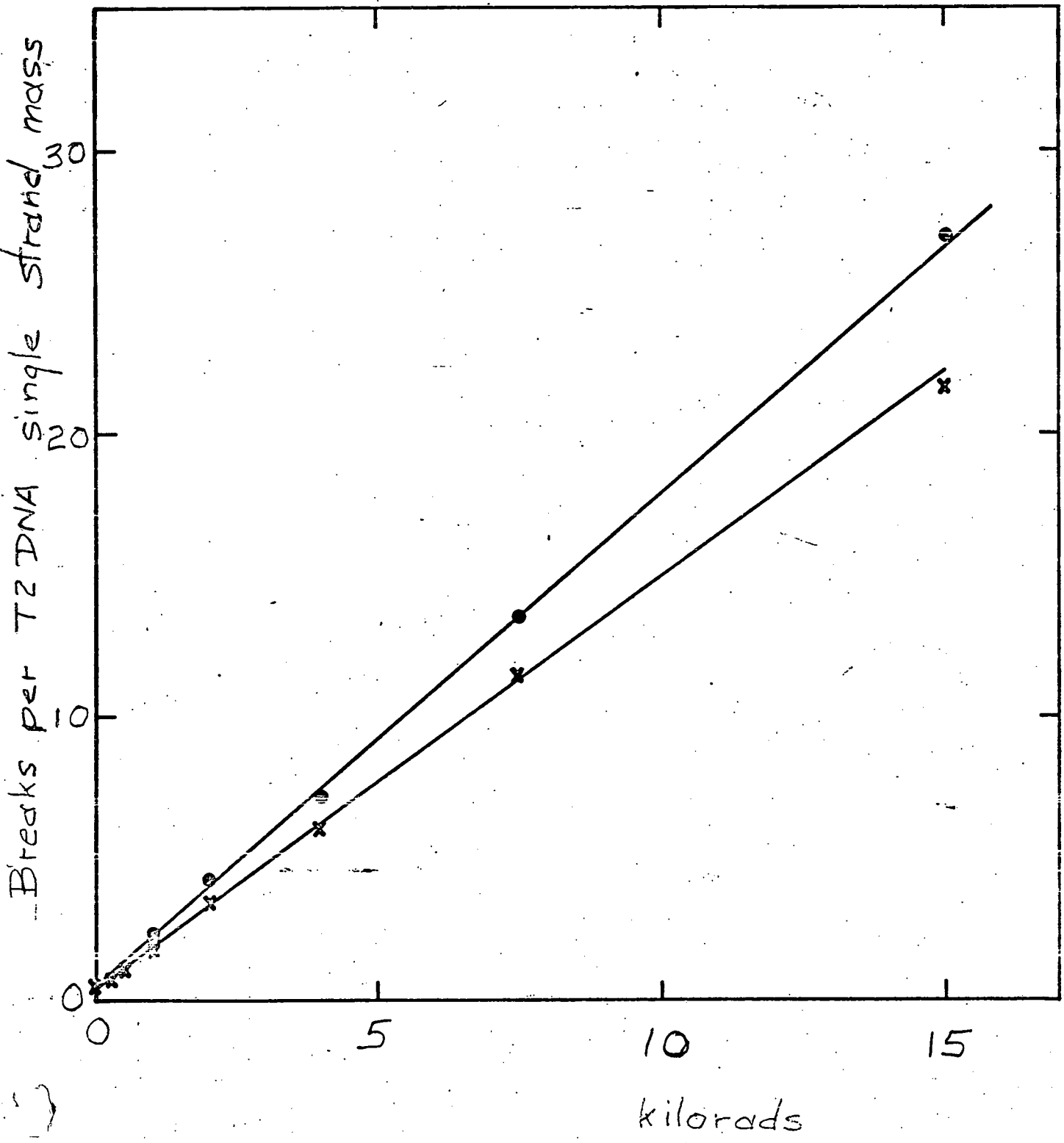
Breaks per T2 DNA single strand mass
0
10
20
30
0
5
10
15
kilorads

Fig. 6