ON THE SIZE OF THE DNA IN THE MAMMALIAN CHROMOSOME

STRUCTURAL SUBUNITS

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ABSTRACT Alkaline degradation of mammalian DNA indicates that the molecule exists in the chromosome as an array of structural subunits. The size of the subunit of single-stranded DNA is *circa* 5×10^8 daltons, and it is of sufficient length to contain a number of synthetic units, replicons. The upper size limit of the multicomponent structure is in excess of 10^{10} daltons. Mammalian cells of three different origins have been shown to contain the same basic structural DNA components and these components exist throughout the cell cycle. The nature of the links between the subunits is not known.

INTRODUCTION

Independent lines of evidence suggest that the DNA in the mammalian chromosome occurs as large continuous molecules (1–3) but that replication is mediated via much smaller units—replicons (4–7). Thus, the strong possibility exists that the chromosomal DNA structure is a continuous array of replicons which are joined through either DNA or nonDNA linkages (5, 8).

Investigations concerned with the formation and rejoining of DNA strand breaks following X-irradiation of mammalian cells have shown that the molecular weight of the DNA in the mammalian chromosome is greater than 5×10^8 daltons. Although this molecular weight represented the upper limit which could actually be measured at that time, extrapolation of the radiation data indicated that the DNA might be present as a few discrete molecules or even a single molecule in each chromosome (9). Improvements in experimental technique were necessary before this possibility could receive further support. The experiments to be described here strengthen the general concept that the DNA is contained in the chromosome as a continuous molecule, but they also show that the molecule is composed of structural subunits which are linked together by alkali-labile bonds. The subunits are uniform in size, are present throughout the cell cycle, and are similar in magnitude from one mammalian cell to the next. There is also a limited amount of evidence that the structural units are themselves an echelon of smaller units which begin to approach the size estimated for the replicon in recent studies (4, 7). Radiation experiments which con-

firm the presence of the subsidiary arrays and quantify their components will be presented at a later date.[1]

In order to measure sedimentation profiles of cellular DNA with currently available ultracentrifugation methods, it is necessary to render the DNA radioactive. The basic tenet which is operative throughout this work is that the level of radioactivity employed for labelling the cellular DNA and the amount of labelling achieved should not interfere with cellular functions, as far as it is possible to determine by using common biological parameters.

MATERIALS AND METHODS

Labelling of Cells

Chinese Hamster Ovary (CHO), the S/S mutant of the murine leukemic lymphoblast L5178Y (10) and HeLa cells were maintained in continuous culture. Synchronous suspensions of CHO cells (mitotic index 94 ± 2%) were obtained by the modification of the shaking technique of Terasima and Tolmach (11) described by Tobey, Anderson, and Petersen (12). Cellular DNA was labelled by treating the cultures for 1.5–2.0 divisions with thymidine-^{14}C (0.01 μCi/ml, 54 mCi/mmole). At this level of radioactivity, no increase in chromosome aberration frequency, or changes in cloning efficiency or growth rate, could be detected. A significant increase (10%) above the spontaneous chromosome aberration rate only occurred when the radioactivity was increased threefold above the experimental level.

Lysis of Cell Suspensions

Suspensions of cells obtained by shaking, by trypsination where necessary, and from single cell cultures were chilled to 0°C, collected by centrifugation, washed in nonradioactive medium, then in physiological saline, and finally resuspended at a concentration of 4×10^5 cells/ml in physiological saline. For the majority of the experiments performed with the zonal rotor, cell concentrations up to 10^6/ml were used.

Centrifugation in Alkaline Sucrose Gradients — SW25.1 and SW27 Swing-out Rotors

Linear 30- and 36-ml 5–20% sucrose gradients containing 0.1 M NaOH, 0.9 M NaCl, and 0.01 M EDTA were prepared at room temperature (20 ± 3°C) in the centrifuge tubes of the Beckman SW25.1 and SW 27 rotors, respectively (Beckman Instruments, Inc., Palo Alto, Calif.). A 0.5 ml aliquot of 0.5 M NaOH, 0.1 M EDTA was gently layered onto the gradient. This was followed by 0.5 ml of the cell suspension and the whole preparation was then kept at room temperature for the requisite storage times. After centrifugation at 20°C, the tubes were pierced at the base and 31 or 37 1-ml fractions were collected, usually through the base. However, this method proved to be inadequate at very high molecular weights and in those cases collections were achieved from the top of the tube using 40% sucrose as the displacement medium. When high resolution was necessary, ½-ml and even ¼-ml fractions were collected following the appropriate reduction in the lytic and cell suspension volumes.

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[1] Lett, J. T., and C. Sun. Manuscript in preparation.

Centrifugation in Alkaline Sucrose Gradients — Titanium Zonal Rotor with Reorienting Gradients

A titanium zonal rotor (B *XXV*-Ti in the Oak Ridge classification) which had been specially modified for operation at low shear rates (N. G. Anderson, private communication) was loaded at rest with a 1430 ml 10–30% sucrose gradient containing 0.2 M NaOH, 1.8 M NaCl, and 0.01 M EDTA and programmed to be linear with radius under centrifugation conditions. This was followed by a 10 ml lytic zone (1.0 M NaOH, 1 M NaCl, and 0.1 M EDTA), by a 20-ml saline wash, by a 10-ml cell suspension (0.14 M NaCl or 0.25 M sucrose), and finally by an 80-ml saline overlay (0.14 M). After the required storage period, the rotor was accelerated (in most experiments) uniformly from rest to 450 rpm in a period of 30 min with a special drive unit and then accelerated normally with the centrifuge drive to the operating speed. In certain cases shorter acceleration times were used. These are indicated in the text. The details of the reorienting system will be described elsewhere. After centrifugation, the rotor was decelerated to 4000 rpm and unloaded at that speed to give 39.5 fractions of 40 ml capacity. 10-ml fractions were collected when higher resolution was needed. The reorientation does distort the gradient but DNA sediments linearly through it. With this experimental procedure the rotor operates with an 80 ml air gap.

Measurement of Radioactivity

The cellular DNA was precipitated from the fractions, together with hydrolysed trout sperm added as carrier (final concentration, 0.2% wet weight), with an equal volume of ice-cold 1 M perchloric acid (PCA). After storing at 0°C for 30 min, the pellets were collected by centrifugation and the DNA extracted with 0.5 M PCA at 80°C for 30 min. After further storage at 0°C for 30 min, the suspensions were centrifuged and aliquots of the supernatants measured in a liquid scintillation counter.

Irradiation

Suspensions of cells in physiological saline were irradiated aerobically at 0–4°C with 250 kvp X-rays (0.25 mm Al) at a dose rate of 3.7 krad/min.

RESULTS AND DISCUSSION

The initial experiments concerned with the formation and rejoining of DNA strand breaks in mammalian cells following X-irradiation were conducted with a Beckman SW50 swing-out rotor (Beckman Instruments, Inc.) (9). Abnormal sedimentation behavior was observed when the X-ray dose was below 5 krad. The weight-average molecular weight of the DNA from aerobically irradiated cells was then *circa* 3×10^8 daltons. At doses where the sedimentation was normal, the profile indicated the random distribution of molecular weights to be expected from radiation damage; but as the X-ray dose was reduced, a point was reached where the DNA sedimented as a rapidly moving band(s) of narrow distribution and the size of the DNA from unirradiated cells could not be determined.

Within the requirements that the level of radioactivity should not produce cellular defects and the need for an adequate radioactive count, it was posible to show that the onset of abnormal sedimentation was dependent upon cell concentration. Since

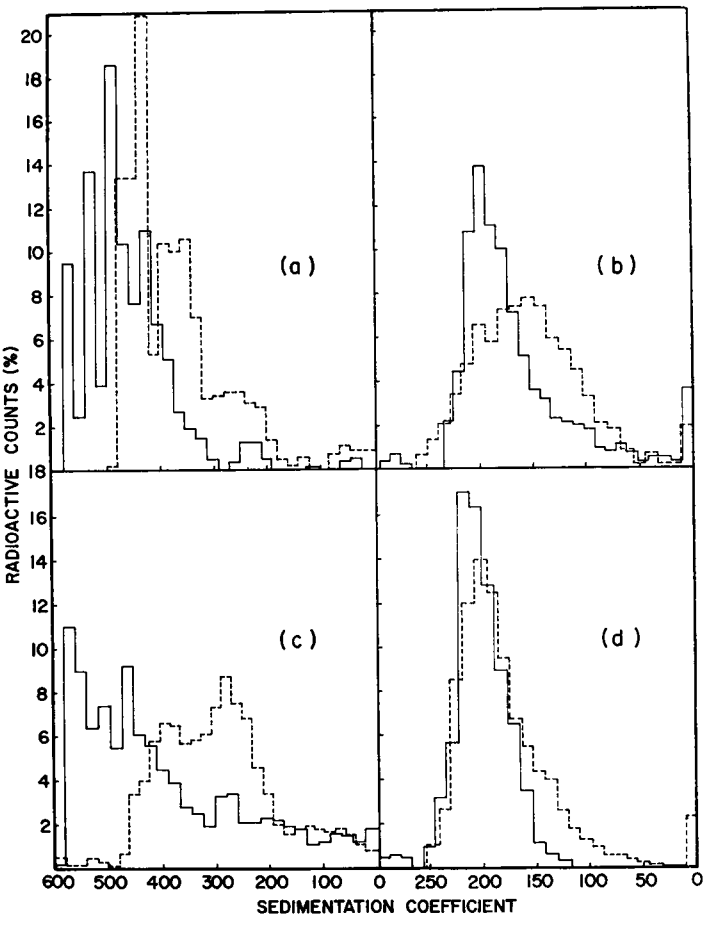

FIGURE 1 DNA sedimentation profiles from CHO cells using the SW25.1 rotor. Asynchronous cells: (*a*), —— 1 hr and ----- 1½ hr lysis; (*b*), —— 9 hr and ----- 18 hr lysis. Mitotic cells: (*c*), —— 1 hr and ---- 2 hr lysis; (*d*), —— 3 hr and ----- 6 hr lysis.

the amount of DNA (minimum radioactive counts) was the limiting factor, the problem was investigated further by keeping the cell number constant and employing rotors which could accommodate increased lytic and cell suspension volumes. Use of the SW25.1 rotor with 1.5×10^5 cells (1 μg DNA) gave sedimentation profiles which tended toward a random size distribution with X-ray doses as low as 500 rad but only when the lysed suspension of irradiated cells was kept for long periods of time on the top of the gradient before centrifugation. The possibility now arose that a modification of the lysis conditions could be utilized for the determination of the molecular weight of the DNA from unirradiated cells. This was enhanced when it was found that structures which represent the rapidly sedimenting components could be disrupted in the presence of the chelating agent ethylene diamine tetracetate, EDTA.

Sedimentation profiles obtained after storing lysed suspensions of asynchronous and mitotic CHO cells for different times in NaOH-EDTA on the top of the alkaline sucrose gradients prior to centrifugation are shown in Fig. 1. A progressive change toward smaller sedimentation coefficients occurs with increasing length of storage. The times necessary for these processes to occur are dependent upon EDTA concentration, pH, temperature, and cell concentration but such studies will not be reported here. One set of conditions was chosen for the majority of the present experiments (with a small variation in the case of the zonal rotor) and this was a compromise between the time of storage before centrifugation, the kinetics of DNA degradation, and centrifugation time. Similar conditions have been used by Sambrook, Westphal, Srinivasan, and Dulbecco (13).

Although the kinetics of the degradation process have not been studied in detail, there is little difference in the general breakdown pattern of the DNA from cells which were in M, G_1, S, or G_2 at the time of lysis. The sedimentation profiles exhibit essentially three main features. During the first few hours there is a degradation (hydrolysis?) through a transient series of DNA components until a mixture of 208S and 165S components is obtained. The second stage of the process (at least temporally) is the slow conversion of the 208S into the 165S component. This is accompanied to some extent, and finally followed by, a process which is difficult to distinguish from random degradation. The profiles in Fig. 1 represent situations in the middle of the degradation process. Certain obvious questions immediately present themselves. What are the upper limits of sedimentation coefficients which can be obtained at very short storage times? Are components liberated at long storage times which are smaller than the 165S component but which are obscured under the general blanket of the random degradation? Is the degradation pattern similar in all (mammalian) cells? Are all the components single strands? Attempts will be made to answer these questions by the experiments which follow, but before this can be done the manner in which sedimentation coefficients and molecular weights have been assigned to the molecular species must be described.

Estimation of Sedimentation Coefficients and Molecular Weights

Single-stranded viral DNA sediments linearly through a linear 5–20% alkaline sucrose gradient in the SW50 rotor (14, 15). The same is true for T_4 bacteriophage DNA and the 208S subunit, for example, in the gradients and rotors employed in this work. Fig. 2 shows the results obtained in the SW25.1 and SW27 swing-out rotors. Calibration of the rotors can now proceed (14, 15) using equations of the type $s_{20,w} = (k\,d)/(\omega^2 t)$, where d (cm) is the distance moved in time t (hr) at a centrifuge speed of ω rpm and k is the calibration constant However, it will be seen that d is concentration dependent and this introduces a difficulty. Both the calibration of the rotors and the use of an equation relating sedimentation coefficient to molecular weight depend upon data obtained in previous investigations (14–16) where a con-

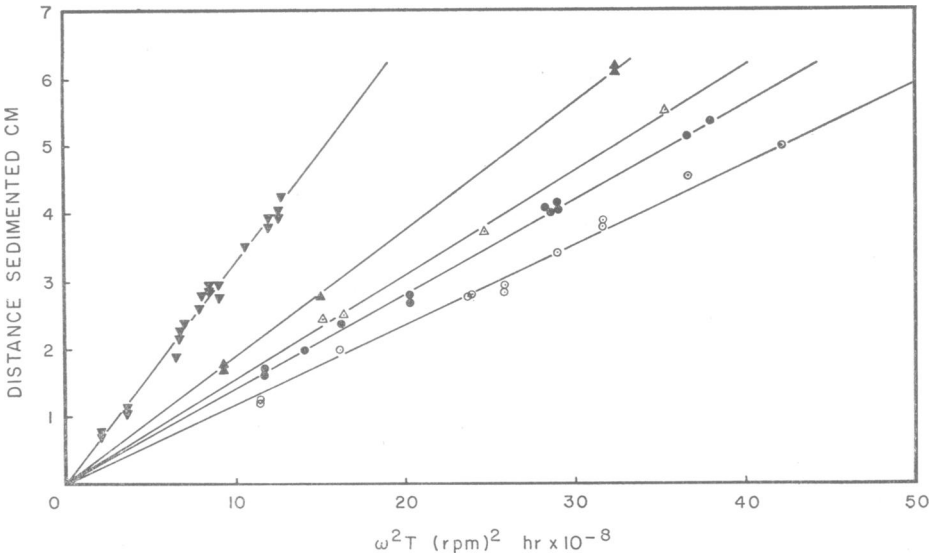

FIGURE 2 Sedimentation of single strands of T_4 bacteriophage DNA and the 208S subunit. SW25.1 rotor: bacteriophage DNA, ⊙, 50 μg/0.5 ml; ●, 1–2 μg/0.5 ml; ▼, 208S subunit, 1–2 μg/0.5 ml. SW27 rotor: bacteriophage DNA, △, 50 μg/0.5 ml; ▲, 1–2 μg/0.5 ml.

centration dependence was either assumed to be absent or not investigated. Abelson and Thomas (15) obtained a value of 71.1 S for the sedimentation coefficient of single strands of T_2 bacteriophage DNA when employing DNA concentrations of "10 μg or less in 0.1 ml" in the SW50 rotor. The analogous situation in the SW25.1 and SW27 rotors would be 50 μg in 0.5 ml (excluding the lytic volume) which is not possible with mammalian DNA because of the high viscosities encountered. Therefore, the following assumptions have been made.

(*a*) Similar concentrations dependencies occur with single strands of DNA greater in size than those from T_4 (T_2) bacteriophage. Such is the case when the 208S subunit is sedimented in the zonal rotor which has sufficient capacity to accommodate the necessary variation in DNA concentration. Larger components could not be studied effectively because the degradation kinetics are concentration dependent.

(*b*) Calibration can be achieved with T_4 bacteriophage DNA at a concentration of 50 μg in 0.5 ml by using a sedimentation coefficient of 71.1 S. This gives a calibration constant in the SW25.1 rotor of 6.0×10^{10}.

(*c*) The molecular weight of mammalian DNA can be obtained from the sedimentation coefficients so evaluated by the use (extrapolation) of Studier's equation (16),

$$s_{20,w} = 0.0528\ M^{0.400}.$$

It is important that these assumptions and possible future reevaluation of the calibration constants be borne in mind in the discussions which follow.

Sedimentation Coefficients in the Range 160–400S

Notwithstanding the assumptions implicit to the calibration of the rotors, the smallest subunit which could be determined unambiguously had a sedimentation coefficient of 165S. When the appropriate lysis conditions were used, the position of this subunit and that assigned the value 208S could be located accurately from simple one- or two-component sedimentation profiles. In other situations the profiles were multicomponent or contained a background of random degradation. The sedimentation coefficients of peaks present in the DNA profiles from CHO cells are given in Table I where the accuracy of resolution (the same applies to Table II) is ± one fraction. Depending on the sedimentation distance, this represents an experimental error of ±3 to ±6%.

Although it was easy to identify peaks in multicomponent profiles, their accurate location was difficult. Frequently it was only possible to determine the position of one peak in each profile with the required resolution.

This situation can be explained by reference to Fig. 1. All the sedimentation values will be approximate because they cannot be read accurately from the figure. The profile following the 1 hr lysis in Fig. 1 (*a*) has four distinct peaks at 560S, 525S, 490S, and 435S. In the 1.5 hr profile, however, only one distinct peak occurs—at 440S; but there is the definite suggestion of further components at the leading edge of the profile, 470S, and at 395S and 355S. If a line is projected vertically from Fig. 1 (*a*) to 1 (*c*) at 470S, it will be seen that a 470S component is distinct in the 1 hr profile and is perhaps also present at the leading edge of the 2 hr profile. A similar projection at 395S shows that components with this sedimentation coefficient may be present in the 1.5 hr and 2 hr profiles in Fig. 1 (*a*) and 1 (*c*), respectively. Another projection at 280S indicates that a peak is almost certainly present in both profiles in Fig. 1 (*c*) and may also be present in the 1.5 hr profile in Fig. 1 (*a*).

On the basis of the terminology outlined above, the 1 hr profile in Fig. 1 (*a*) was considered to contain four resolvable components while the 1.5 hr profile contained only one, although three other peaks could be identified. The profiles in Fig. 1 (*c*) contained only one resolvable peak each, although other components could be identified. Sedimentation coefficients are not given in Tables I and II unless the peaks can be resolved in this manner, nor is a single value given unless it has been *identified* in other profiles on at least two occasions. For example, in the 4 hr lysis experiments shown in Table I, a 308S value is given only once but components with sedimentation coefficients *circa* 310S were identified in other profiles. When peaks which can be considered as representing components with the same sedimentation coefficients have been resolved in separate experiments, they are given separately in Table I.

A number of experiments were needed to get reliable representation of the components present in the 160–400S range. As it seems probable that the majority of such components are contained in Tables I and II, molecular weights have been assigned to the macromolecular species. These molecular weights are given in Table II. The values were obtained by extrapolation of Studier's equation. Justification for the extrapolation will follow later.

TABLE I

SEDIMENTATION COEFFICIENTS PRESENT IN THE DNA PROFILES FROM CHO CELLS FOLLOWING DIFFERENT LYSIS TIMES

Storage time	Sedimentation coefficients, S	No. of Expt.
hr		
0*	833 787 775 664 630 595 552 528 509 490 470 444 434 414 392 388 388 378 371 351 338 298 275 274 268 218 214 203 198 192 173 170 169 167 165 163 160 158 145 142 122 108	2
0.1*	665 619 525 434	1
0.2*	654	1
0.3*		1
0.5*		1
0.7		1
0.8		1
1.0* (M)‡		3
1.5 (M)		1
2.0* (M)		6
3.0 (M)		3
4.0* (M)		13
5.0 (M)		2
9.0		1
12.0		1
18.0 (M)		2

* Indicates one or more high resolution experiments.

‡ (M) includes experiments with mitotic cells.

TABLE II

SEDIMENTATION COEFFICIENTS PRESENT IN THE DNA PROFILES FROM CHO, L5178Y, AND HELA CELLS

Cell type	Chromosome No.	Rotor	Mean values of sedimentation coefficients, S																								
CHO	21	SW25.1	833	781 ±6		661 ±7	625 ±6	595	555 ±4	520 ±11	490	470	439 ±5	417 ±3	389 ±3	377 ±4	351	338 ±7	303 ±5	276 ±11	239	205 ±13	165 ±7	144 ±2		122	108	
L5178Y	40	BXXV-Ti	857	788	741 ±17		641 ±8	598 ±7	555 ±10		491 ±9	463 ±2		417	397 ±4				315	280 ±8		216 ±8	168 ±6		131	120		87
HeLa	48+	SW25.1						608			489 ±2	469 ±3			397 ±8	377				267 ±8	246	208 ±9	162 ±4	137		117	101	89
Over-all mean values			845 ±12	783 ±8	741 ±17	661 ±7	633 ±14	601 ±7	555 ±10	520 ±11	490 ±10	466 ±5	439 ±5	417 ±3	394 ±11	377 ±4	351	338 ±7	307 ±8	275 ±13	243 ±4	208 ±13	165 ±7	141 ±4	131	120 ±3	105 ±4	88 ±1
MW ($\times 10^{-6}$) from Studier's equation															48.1	43.1	36.0	32.8	25.8	19.6	14.4	9.75	5.45					

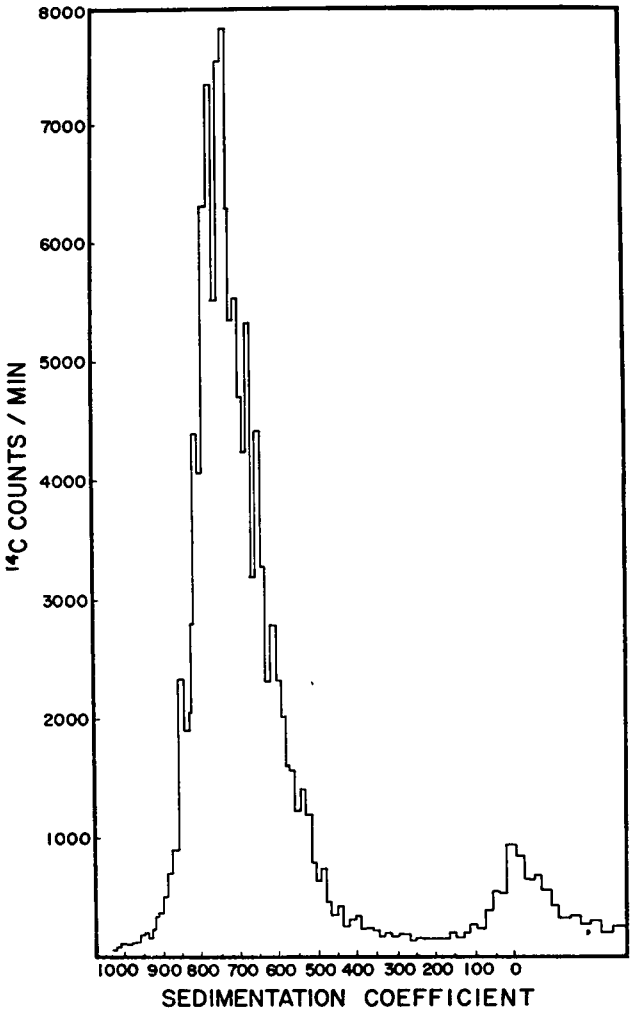

FIGURE 3 Sedimentation profile from asynchronous L5178Y cells in the XXV-Ti rotor using rapid gradient reorientation and high resolution. Lysis time, 20 min.; reorienting time, 10 min to 450 rpm.

Sedimentation Coefficients above 400S — High Resolution

The ordered sequence of the DNA degradation in alkaline EDTA suggests that the largest macromolecular components can be evaluated following short lytic times. Most of the molecular weights assigned to the sedimentation coefficients in the range 160–400S in Table II *can* be fitted (with an accuracy of ± 5%) to a multiple of a structural subunit of the size 5.2×10^8 daltons. Above this range the differences in molecular size between such multiples give differences in sedimentation coefficients which fall within experimental error, and high resolution was necessary to avoid

spurious averaging. Moreover, the sedimentation times are long compared to the lytic times, which strengthens the possibility of change in structure, and hence, sedimentation coefficient during centrifugation.

As the sedimentation coefficient increases above 400S, the viscosity of the DNA becomes pronounced even with 1 μg quantities used in the SW25.1 and SW27 rotors. The viscous zones occlude denatured material (protein?) and cause it to sediment with the DNA. Collection through the base of the tube must be abandoned because of the flow properties of the viscous layers. Even with the employment of high resolution (experimental error $\pm 2\%$) and collection through the top of the centrifuge tube, the sedimentation coefficients greater than 400S given in Tables I and II must be treated as provisional. Added caution is also necessary because of the possibility that the components assigned sedimentation coefficients above 400S are double helices. Extrapolation of the unwinding times observed by Davison (17) to moleculae sizes in the range 10^9–10^{10} daltons would indicate that several hours storage arr necessary to achieve denaturation of the double helix.

The zonal rotor represents a solution to the difficulties of viscosity, concentration-dependent characteristics of the type encountered with T_4 bacteriophage DNA, and potential for high resolution. One demerit, however, is the need for gradient reorientation resulting in an inherent lytic period which cannot be eliminated. Experiments involving the minimum reorienting times compatible with low shearing rates are in progress. The profile obtained from one such experiment is shown in

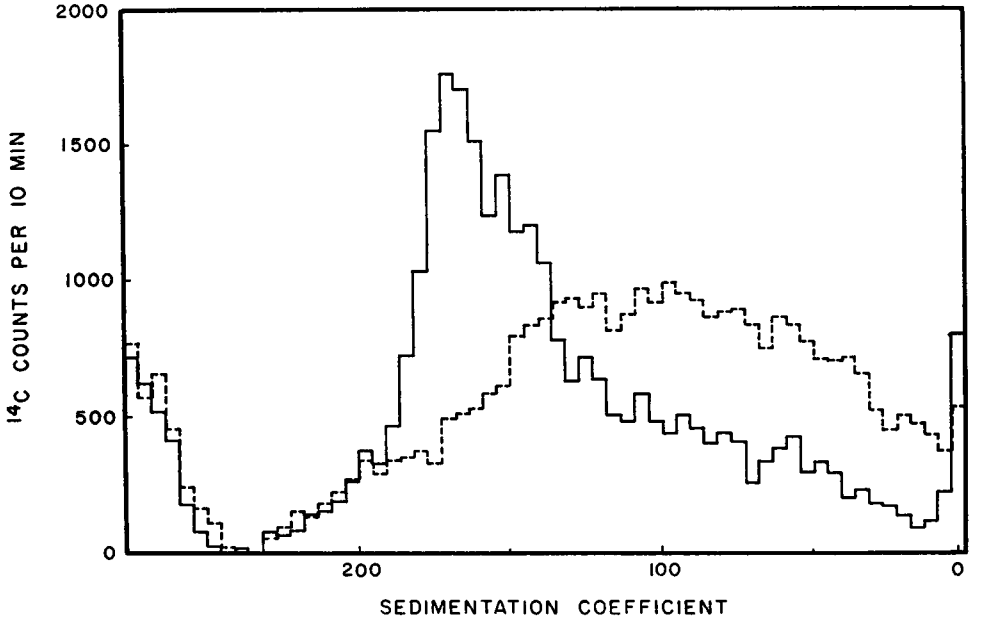

FIGURE 4 Extensive degradation of mitotic CHO cells. Profiles from the SW25.1 rotor at high resolution. Lysis time 20 hr ——; lysis time 60 hr -----.

Fig. 3. Shorter lytic times have been used and components with sedimentation coefficients in excess of 1000S have been observed.

Sedimentation Coefficients below 160S—Random Degradation

The detection of monodisperse components in the presence of extensive random degradation also requires high resolution. For example, the 18 hr degradation profile in Fig. 1 can be fitted by random distribution of molecular weights even though much of it consists of the 165S and 208S subunits. In about 10% of the profiles, it was possible to *identify* components with sedimentation coefficients below 165S. They are recorded in Tables I and II. Usually the profiles obtained after long storage times had the appearance of those shown in Fig. 4, which contains only the suggestion of smaller components under what is seemingly the random degradation of the 165S subunit. In general, the profiles were poorly defined and not reproducible.

All the long term degradation high resolution experiments were performed with mitotic cell populations to avoid the complications which could arise from partially synthesized DNA.

The Nature of the Subunit Links

The subunits may be linked by DNA or nonDNA bonds. Linkers composed of protein can in theory be observed by the use of labelled amino acids. Thus far, the success of this method in ascertaining protein levels in the sedimentation profiles has been the same as that observed previously, i.e., an upper limit of 0.5% (9). Since high levels of labelled amino acids produce severe cellular abnormalities and even kill the cells, the potential for examination of the subunit links lies with the high capacity of the zonal rotor.

Mammalian Cells of Different Genetic Origin

In order to test the hypothesis that the DNA from the chromosomes of mammalian cells of different genetic origin may have the same basic multicomponent structure, the sedimentation profiles of the DNA from CHO cells, L5178Y murine leukemic lymphoblasts, and HeLa cells were compared after controlled degradation. The results obtained are given in Table II. Experiments with L5178Y and HeLa cells were more limited than those with CHO cells and some of the identifiable components present in the 165–400S range are not recorded in Table II (see previous discussion on page 278). The cell lines were chosen because of differences both in origin and chromosome number viz. 21, 40, and 46 plus, respectively. With the described levels of resolution, the profiles were identical once the degradation reduced the component size below 400S. Since there are DNA components with the same sedimentation coefficients from each cell type, the over-all mean values have

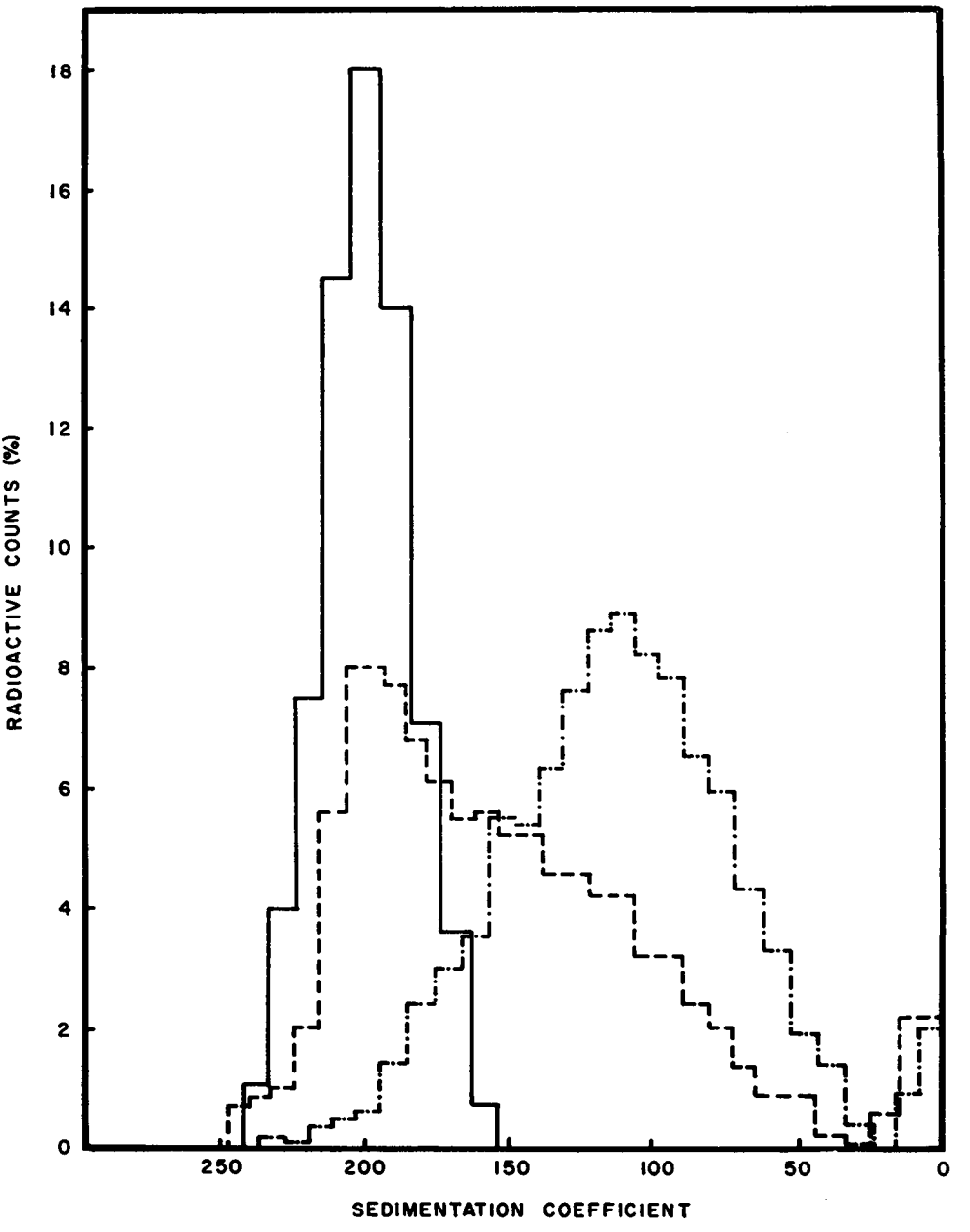

FIGURE 5 Change from monodisperse toward random distribution following X-irradiation of the 208S subunit. X-ray dose, 0 rad ——, 1600 rad – – –, 3100 rad —·—·—.

also been given in Table II and it is to these mean values that the molecular weights have been assigned. Of course, differences in base ratios would result in small differences in molecular weights even if the polynucleotide lengths were identical.

Existence of Single Strands in the Sedimentation Profiles

X-rays produce single breaks in mammalian DNA (L5178Y cells) with an efficiency of 70 ev/break (9). Recent estimates with CHO cells using the rotor calibration described above give a figure of 55 ev/break.[1] On the other hand, double breaks, that is adjacent polynucleotide breaks in both strands of the twin helix, are produced ten times less efficiently (18, 19). The effect of X-irradiation on the closely monodisperse distribution associated with the 208S subunit is shown in Fig. 5. The percentage of unbroken molecules can be calculated from these profile changes and from similar effects on the profile of the 165S subunit. The efficiency of strand breakage which is obtained from the D_{37} dose (i.e. the dose at which 37% of the component molecules remain unbroken) is 65 $\pm$ 5 ev/break for each subunit. The calculation of the efficiency with which X-rays produce single strand breaks requires a knowledge of the molecular weight of the DNA subunits. The internal agreement between the values obtained for the efficiency of breakage from the destruction of the monodisperse distributions of the 165S and 208S subunits and from random distributions in the size range where only limited extrapolation of Studier's equation is necessary (9, 18) must imply that the lengthy extrapolation for molecular weights up to 10^9 daltons is substantially correct and that the components are single strands of DNA.

Recent estimates (with Chinese Hamster and L5178Y cells) suggest that the size of the replicon lies within the range 2–7 $\times$ 10^7 daltons (4, 7) which would require sedimentation coefficients below 165S where the sedimentation data are equivocal. Nevertheless, the possibility does exist (Tables I and II) that DNA molecules of comparable sizes are present in the degradation profiles and confirmation will follow from the radiation data to be presented later.[1] Autoradiographic studies have indicated a spectrum of replicated lengths of DNA in the chromosome from less than 30 μ (4) to 2 cm (1). Formal correlation with the replicon studies would be premature because in those experiments the high levels of radioactive thymidine employed caused pronounced cellular damage (4) so that a proportion of the strand lengths observed may represent repair synthesis rather than normal replication synthesis. One of the principal objectives in the present work has been to avoid, where possible, cell damage from radioactive precursors. The smallest structural, as opposed to synthetic, unit of single-stranded DNA that can be ascertained at the present time has a molecular weight of 5.5 $\times$ 10^8 daltons while the upper limit of the subunit array is in excess of 10^{10} daltons.

The foregoing data support the concept that the DNA in the mammalian chromosome exists as a long continuous array of subunits. The nature of the links between the subunits is not known. They could be nonDNA in nature, e.g. protein or peptide, or certain types of DNA bonds located at specific loci could themselves be susceptible to chemical or enzymic attack. Certainly the multicomponent structures can behave as continuous DNA molecules. Flexibility about the links, or a facile

system for making and breaking them *in situ*, could provide the mechanism whereby the arrays can be accommodated within the compact chromosomal structure and still provide initiation points for DNA synthesis. It could also account for crossing-over in chromosomes. A facile making-and-breaking system which would allow the elimination of a subunit or a multiple unit could also admit and retain viral DNA. The uncertainties involved in the measurement of sedimentation coefficients and molecular weights does not permit quantitative description of the degradation scheme at this time. They do, however, serve to underline why CHO cells have been used as the main source of mammalian DNA in these experiments. The chromosomes of the CHO cell can be separated into six fractions (20) one of which is composed of very small chromosomes. The DNA from these chromosomes should provide an excellent size marker 2–3 orders of magnitude above the range used by Studier (16) and be a starting point for the larger chromosomes. Preliminary experiments along these lines have been encouraging.

The larger the DNA component, the smaller is the dose needed to break a given percentage of the molecules; hence, the potential exists for detecting DNA damage at very small, i.e. biological, doses. Answers have already been obtained with doses as low as 10–50 rad. This theme, together with some of the experimental difficulties involved, is developed elsewhere.[2]

Note Added in Proof. The concept of structural DNA components must be extended to all living organisms — not least from the standpoint of evolution. Analogous, time-dependent degradation of chromosomal DNA through a series of components has already been observed in several genera of bacteria and the studies are being further extended to plants, fishes, etc.

The first serious effort to undertake this work was made after the discussions between Dr. J. T. Lett and Drs. P. M. Corry and A. Cole of the M. D. Anderson Hospital, Houston, Texas when attempts were made to correlate their data (19) from neutral gradients with the earlier results (9) from alkaline gradients.

Our thanks are expressed to Dr. N. G. Anderson and his colleagues at Oak Ridge for the loan of the B XXV-Ti zonal rotor, for the construction of the slow speed reorienting device, and in numerous other ways.

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