

# THE DNA OF *CAENORHABDITIS ELEGANS*

J. E. SULSTON AND S. BRENNER

*Medical Research Council Laboratory of Molecular Biology,  
Hills Road, Cambridge, CB2 2QH, England*

Manuscript received December 10, 1973

## ABSTRACT

Chemical analysis and a study of renaturation kinetics show that the nematode, *Caenorhabditis elegans*, has a haploid DNA content of  $8 \times 10^7$  base pairs (20 times the genome of *E. coli*). Eighty-three percent of the DNA sequences are unique. The mean base composition is 36% GC; a small component, containing the rRNA cistrons, has a base composition of 51% GC. The haploid genome contains about 300 genes for 4S RNA, 110 for 5S RNA, and 55 for (18 + 28)S RNA.

THE small nematode, *Caenorhabditis elegans*, is the subject of extensive genetical study in this laboratory (BRENNER 1974). During the course of this work it became apparent that the number of genes detectable by mutation was surprisingly small, and it therefore became necessary to determine the haploid DNA content of this organism. We included a study of the renaturation kinetics of the DNA because of the special significance attached to the content of unique sequence DNA. We show here that *C. elegans* has a haploid DNA content about 20 times that of *E. coli* and that 83% of the DNA sequences are unique. We have also measured the number of sequences corresponding to tRNA and rRNA by DNA-RNA hybridization.

## MATERIALS AND METHODS

*Media:* 1. S medium: 1 ml of a saturated ethanolic solution of cholesterol is added to 1 litre of S buffer (0.1 M NaCl, 0.05 M potassium phosphate, pH 6.0). After autoclaving, sterile solutions of the following are added in order: 10 ml M potassium citrate (pH 6.0), 3 ml M CaCl<sub>2</sub>, 3 ml M MgSO<sub>4</sub> and 10 ml trace metals solution (EDTA 5 mM, FeSO<sub>4</sub> 2.5 mM, MnCl<sub>2</sub> 1 mM, ZnSO<sub>4</sub> 1 mM and CuSO<sub>4</sub> 0.1 mM).

2. SLP medium: S medium with potassium phosphate reduced to 0.01 M and supplemented with 0.04 M imidazole hydrochloride, pH 6.0.

3. Low phosphate medium: 1 litre contains 5 g NaCl, 1.5 g KCl, 1 g NH<sub>4</sub>Cl, 2 g Difco vitamin-free casamino acids, 2 g Difco Bactopeptone and 12.1 g Tris base; adjusted to pH 7.4 with HCl.

4. Phosphate buffer: Equimolar mixture of NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>.

5. SSC: 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0.

*Culture methods:* The general methods for maintaining and handling *C. elegans* are described by BRENNER (1974).

Mass cultures are grown on dense suspensions of bacteria in S medium. *Escherichia coli*, *Proteus mirabilis* and *Serratia marcescens* have all been used successfully, but *Serratia* undergoes lysis and contaminates the product. The bacteria are grown to saturation in broth, cen-

trifuged and resuspended in S medium at a concentration of 20 g wet weight per litre. One litre Roux bottles, fitted with open-ended spargers, are half filled with the suspension, inoculated with about  $2 \times 10^4$  worms washed off plates and the cultures vigorously aerated at 20°. After about five days the cultures clear, and the nematodes are allowed to settle at 4° overnight. They are resuspended in cold 35% w/v sucrose and immediately centrifuged in a swinging bucket at 3,000 r.p.m. for 5 minutes. Bacteria and debris sediment and the nematodes, which float to the top, are removed and washed twice with 0.1 M NaCl. The yield of nematodes is about 5 gm wet weight per litre of culture. Bacterial contamination, as judged by caesium chloride gradient centrifugation of the DNA, is not detectable when *E. coli* is used, but amounts to about 1% to 2% with *S. marcescens*.

In some experiments adults and larvae have been separated as follows. 200 ml of a suspension of worms in 0.1 M NaCl is layered on a sucrose gradient (1–5% in 0.1 M NaCl) in a 2-litre measuring cylinder. After 1 hour at 4° a mixture of L1 and L2 larvae is recovered from the middle third of the gradient while adults have settled to the bottom. L3 larvae can be isolated from the lower third of the gradient by a second sedimentation. L4 larvae are not found in cleared liquid cultures.

**DNA extraction:** The nematodes are frozen in liquid nitrogen and then ground to powder which is gently stirred into 20 volumes of a solution containing 0.1 M NaCl, 0.1 M EDTA, 0.05 M Tris-HCl (pH 8.0), 1% SDS and 200 µg/ml of protease (Sigma Type VI). An overnight incubation at 37° is followed by phenol extraction and the nucleic acids from the aqueous phase are spooled into 2 volumes of ethanol (MARMUR 1961). The material is dispersed in SSC, treated with preheated pancreatic ribonuclease followed by self-digested protease, extracted with phenol and spooled again. Any remaining RNA and polysaccharide are removed by fractionation on hydroxyapatite. The yield of DNA is 1–2 mg per g wet weight of nematodes.

**DNA renaturation:** The techniques for DNA renaturation are taken from KOHNE and BRITTEN (1971) and LAIRD (1971). Purified DNA is dissolved in 0.5 M sodium chloride and sheared by passage through a French press at 20,000 psi. The mean  $S_{20,w}$  of the sheared DNA in alkaline solution is about 5.5, corresponding to a single strand length of 350 nucleotides (STUDIER 1965). After incubation in 1 N sodium hydroxide at 37° for 1 hour, the DNA is dialyzed exhaustively against water, freeze dried, and dissolved in 0.12 M phosphate buffer. Aliquots are sealed in glass tubes, heated to 100° for 5 min, and kept at 60° for various times. The products are analyzed by hydroxyapatite chromatography at 60°.

**<sup>32</sup>P-labelled nematodes:** <sup>32</sup>PO<sub>4</sub> is incorporated very inefficiently by nematodes from the medium, even when the phosphate concentration is reduced to the lowest tolerable level. Better results are obtained by growing *E. coli* K12 to exhaustion on <sup>32</sup>P low phosphate medium, and then growing the nematodes on a suspension of the labelled bacteria in SLP medium. *E. coli* strain B must not be used; it is radiation-sensitive and, at the specific activities used, such bacteria form long snakes which cannot be consumed by the worms.

Three methods have been used. 1. Uniform labelling: 1 g of bacteria, containing 15 mC of <sup>32</sup>P, are suspended in 100 ml of SLP medium. 5000 nematodes are added and the culture is aerated at 20° until it clears (about 5 days). The nematodes contain 0.6 mC of <sup>32</sup>P at a specific activity of 30 C/M. 2. Pulse labelling: 0.4 g of bacteria, containing 50 mC of <sup>32</sup>P, are suspended in 100 ml of SLP medium.  $5 \times 10^5$  nematodes (from 130 ml of a standard liquid culture) are added and the culture is aerated at 20° for 16 hours. The nematodes do not reproduce under these conditions. They contain 14 mC of <sup>32</sup>P at a specific activity of 200 C/M. By decreasing the inoculum higher specific activities can be achieved at the expense of total incorporation. 3. Plate method: NG plates (BRENNER 1974) are prepared without added phosphate; the residual phosphate amounts to 10<sup>-4</sup> M. <sup>32</sup>P inorganic phosphate is spread over the surface of the plates, which are then inoculated with *E. coli* K12 and nematodes. The nematodes multiply at specific activities up to 100 C/M.

In order to flush out labelled bacteria from their guts, the nematodes are transferred to small volumes of S medium containing non-radioactive *E. coli* (20 g wet weight/litre). After aeration for 30 minutes, the nematodes are isolated as described above.

**Preparation of <sup>32</sup>P-RNA:** Up to 14 mC of pulse labelled nematodes are suspended in 3 ml of extraction buffer (0.5 M sodium acetate, pH 5.5, 0.002 M MgCl<sub>2</sub> and a trace of phenol). The

nematodes are disrupted by passage through a French pressure cell at 4° and 13,000 psi. Later experiments have shown that degradation of rRNA can be greatly reduced by adding an equal volume of washed bentonite to the nematodes before they are broken. The effluent is shaken vigorously with an equal volume of phenol at 70° for 10 minutes, cooled in ice, and centrifuged. The RNA is precipitated from the separated aqueous phase with ethanol, dried and applied in a small volume of buffer to an 8% polyacrylamide slab gel (LAEMMLI 1970). After electrophoresis, the 4S, 5S and (28 + 18)S regions are eluted, further purified by the same procedure and then finally dissolved in 0.5 ml of 0.01 M Tris-HCl, pH 7.0. The solutions are extracted with ether before measuring their optical density and specific activity.

**RNA-DNA hybridization:** The methods of GILLESPIE and SPIEGELMAN (1965) are followed. DNA is incubated in 1 N sodium hydroxide at 37° for 1 hour, neutralized in 6 × SSC, and loaded onto 10 mm filters. Fractions from caesium chloride gradients are similarly loaded onto 25-mm filters, from each of which four 10 mm filters are punched. Solutions of <sup>32</sup>P-labelled RNA's in 2 × SSC are placed in siliconized 2 × ½ inch flat-bottomed glass vials. Up to 35 filters are stacked in each ml, but the minimum satisfactory volume is 0.2 ml. The vials are incubated at 65° for 14 hours. The filters are washed in 2 × SSC, incubated with 20 µg/ml of preheated pancreatic ribonuclease in 2 × SSC for 1 hour, and washed again with 2 × SSC. After drying, radioactivity is measured in a scintillation counter.

**Caesium chloride density gradient centrifugation:** The method of analytical centrifugation is that of SCHILDKRAUT, MARMUR and DOTY (1962). For preparative gradients, 100 µg of DNA is dissolved in 4 ml of CsCl ( $\rho = 1.71$ ) and the solution is passed through a 26G hypodermic needle at a rate of 0.25 ml/sec. After overlaying with oil, the solution is centrifuged in a Ti50 rotor at 37,000 r.p.m. at 15° for 60 hours. Fractions are collected through a short 1 mm needle.

## RESULTS

**Base composition:** In a caesium chloride gradient (Figure 1) most of the DNA bands at a position corresponding to a base composition of 36% GC (SCHILDKRAUT, MARMUR and DOTY 1962). A small proportion of the DNA bands at a

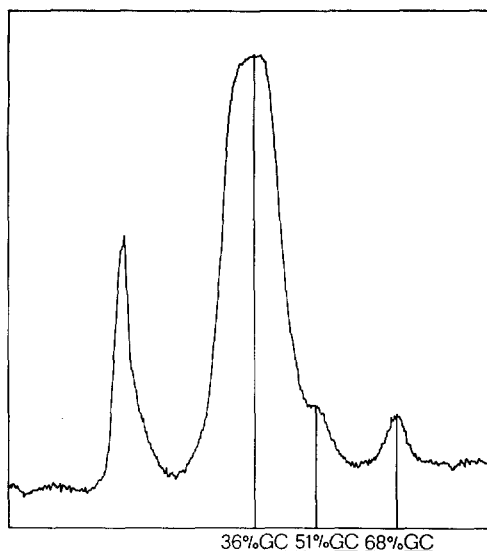


FIGURE 1.—Caesium chloride density gradient centrifugation of DNA, purified to second spool, from *C. elegans* (grown on *P. mirabilis*, 41% GC); marker DNA from *P. aeruginosa*, 68% GC. Beckmann Model E ultracentrifuge, 20 hours, 20°. The gradient has been overloaded in order to display the satellite band.

position corresponding to 51% GC; rRNA hybridizes with DNA from this region of the gradient. The DNA was obtained from nematodes grown on *P. mirabilis* (DNA base composition 41% GC), so that any contaminating bacterial DNA is not visible as a satellite. The material of low density, lying to the left of the main band, is probably polysaccharide. It can be removed by purification on hydroxyapatite; this process, however, reduces the molecular weight of the DNA, so that the satellite is less clearly resolved.

This estimate of base composition was confirmed by direct analysis.  $^{32}\text{P}$ -labelled DNA was completely hydrolyzed to nucleotides by a mixture of pancreatic deoxyribonuclease and venom phosphodiesterase. The products were separated by electrophoresis at pH 3.5. 35.5% of the radioactivity was in dGMP and dCMP.

The base compositions of other nematode DNA's have been determined by BEHME and PASTERNAK (1969). *C. elegans* lies at the lower end of the range.

*DNA content:* We have attempted to estimate the DNA content by chemical methods. A mixture of L1 and L2 larvae was isolated and samples were removed for counting and DNA estimations. Three methods were used. (1) The nematodes, in 1 ml of distilled water, were disrupted by sonication at 0° (Dawe sonifier, level 4, 30 secs), and then precipitated with cold 5% TCA. The DNA in the pellet was assayed as described by BURTON (1968), using purified *E. coli* DNA as a standard. The average DNA content of a nematode was found to be  $1.1 \times 10^{11}$  base pairs. (2) A known amount of *Pseudomonas aeruginosa* DNA was added to a sample of nematodes and the usual DNA extraction procedure was followed as far as the first spool. A sample was centrifuged to equilibrium in a caesium chloride gradient and the amounts of *C. elegans* and *P. aeruginosa* DNA were computed by densitometry. This gave  $0.8 \times 10^{11}$  base pairs as the average DNA content per nematode. (3) The yield of fully purified DNA from larvae corresponded to an average content of  $0.9 \times 10^{11}$  base pairs.

These results show that a value of  $1.0 \times 10^{11}$  base pairs is reasonable for the DNA content of a larva. S. PICKVANCE (unpublished results) has estimated that such larvae contain about 600 nuclei, which agrees with the estimate of SIN and PASTERNAK (1970) for *Panagrellus*. In this species most of the nuclei were shown to be diploid at this stage and this is likely to be true for *C. elegans* larvae as well. This leads to an estimate of about  $1.6 \times 10^8$  base pairs per nucleus and the haploid genome is therefore expected to contain  $0.8 \times 10^8$  base pairs ( $8.8 \times 10^{-14}$  g).

Adult nematodes obtained from liquid cultures contain about fifteen times more DNA than do larvae. The growth of the gonad and the number of developing embryos is sufficient to account for most of this increase.

*Kinetics of renaturation:* In order to determine the sequence complexity of the *C. elegans* genome we studied its renaturation. Figure 3 shows that the DNA can be divided into two components. 17% renatures rapidly and is kinetically heterogeneous, while 83% renatures slowly and is kinetically homogenous. The latter corresponds to unique sequence DNA and, as shown in Figure 2, it has a  $T_m$  only slightly below that of native sheared DNA, as expected for well-matched sequences. The slope of the slow part of the *C. elegans* renaturation curve is 1/24 that of the *E. coli* curve. After correction for the effect of base composition on

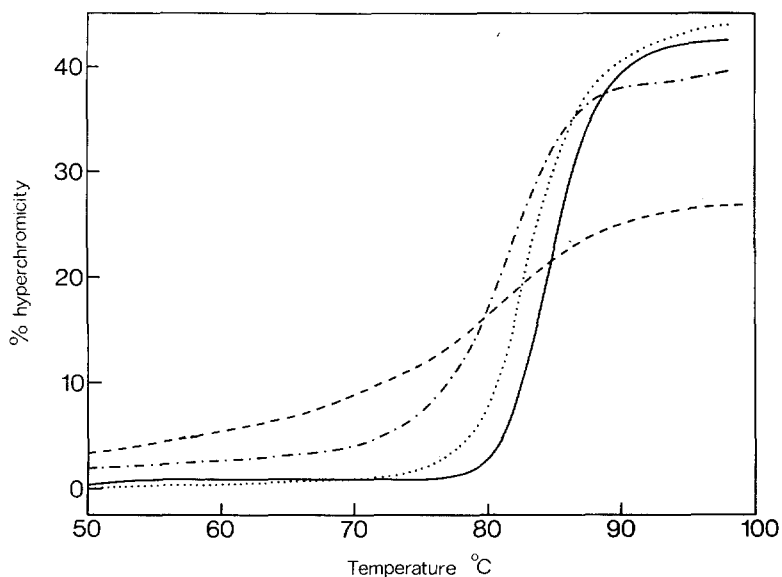


FIGURE 2.—Thermal hyperchromicity of *C. elegans* DNA samples in 0.12 M phosphate buffer, referred to optical density at 25° and corrected for expansion of water. Unicam SP500 spectrophotometer. —: native; .....: sheared; - - - -: renatured to  $C_0t$  10; - · - · -: renatured to  $C_0t$  1000.

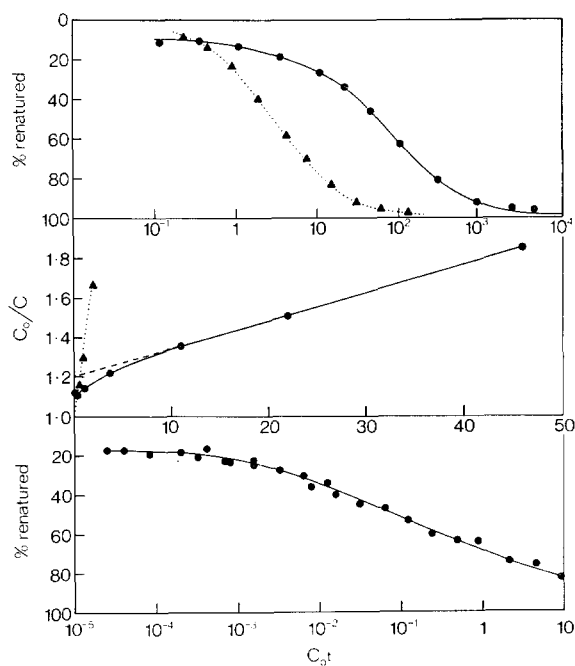


FIGURE 3.—Renaturation of *C. elegans* DNA. (a), (b): *C. elegans* DNA (●—●). *E. coli* DNA is shown for comparison (▲.....▲). (c): Rapidly renaturing component of  $^{32}\text{P}$ -labelled *C. elegans* DNA (18% of the total DNA).  $C_0$  and  $C$  are the concentrations of denatured DNA at zero time and time  $t$  respectively.

renaturation (WETMUR and DAVIDSON 1968), the complexity of the unique component corresponds to  $0.67 \times 10^8$  base pairs, which leads to an estimate of  $0.8 \times 10^8$  base pairs for the haploid genome of *C. elegans*. This is in good agreement with the haploid DNA content measured chemically, and reinforces the conclusion that the slow component is uniquely represented in the genome.

The rapidly renaturing component was isolated from  $^{32}\text{P}$ -DNA by two successive incubations to  $C_{ot}$  15. Its melting curve is broad, indicating considerable mismatching (Figure 2). 80% of this component (14% of the total DNA) is repetitious DNA, consisting of families of sequences repeated from 10 to 10,000 times in the haploid genome. Presumably part of this repetitious DNA is mitochondrial; but since we have not isolated mitochondrial DNA, no estimate of its abundance is possible. The absence of a detectable light satellite in caesium chloride gradients suggests that mitochondrial DNA may only be a minor component of DNA preparations. As in other organisms, the repetitious DNA includes the cistrons for tRNA and rRNA (Table 1).

The remainder of the rapidly annealing component, which corresponds to about 3% of the total DNA, renatures completely at  $C_{ot} < 10^{-7}$ , indicating that the reaction is intramolecular. Such extremely rapidly renaturing or "fold back" DNA has been found in other eukaryotes (BRITTEN and SMITH 1969). The yield of this component varies with the extent to which the DNA is sheared (from 2.5% at 20,000 psi to 6% at 3,000 psi), but is the same independently of whether the DNA is initially denatured by heat or by alkali. The mean strand length, as measured by sedimentation in alkali, is not significantly greater than that of the remaining DNA. The melting curve is very broad. The results rule out cross-linked DNA as a major component of the extremely rapidly renaturing fraction. The same yield of this component is obtained from different caesium chloride density fractions of *C. elegans* DNA. This suggests that it is probably widely distributed throughout the genome.

*Comparison of larval and adult DNA:* In order to test whether there is any difference between germ line and somatic DNA in *C. elegans* we have compared the DNA of immature larvae in which the gonads have not yet developed with that of adults which contain relatively large numbers of germ cells. DNA from L1 + L2 larvae and from adults were each separately renatured in the presence

TABLE 1  
*Results of DNA-RNA hybridization analysis*

RNA	% of genome	No. of copies	DNA %GC*	RNA %GC†
4S	.028	300	38 (broad)	60
5S	.017	110	32	47
(28 + 18)S	.44	55	51	48

\* By density in caesium chloride gradient.

† By alkaline hydrolysis and electrophoresis at pH 3.5.

of a small amount of  $^{32}\text{P}$ -DNA from adults. Figure 4 shows that no differences could be detected.

*Cistrons for rRNA and tRNA:* The numbers of genes corresponding to these RNA's were measured by DNA-RNA hybridization. 4S, 5S and (28 + 18)S RNA fractions were isolated from  $^{32}\text{P}$ -labelled nematodes. The lower molecular weight

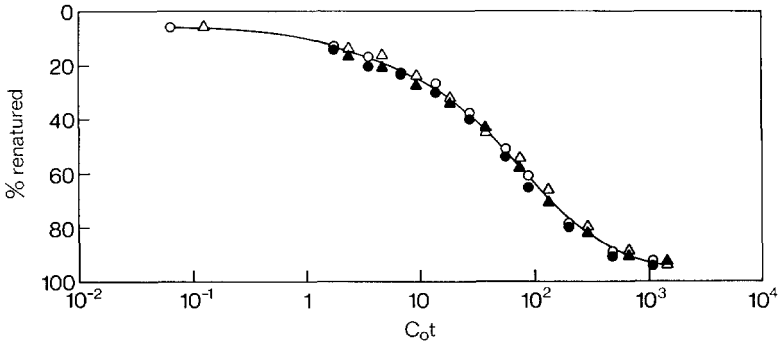


FIGURE 4.—Two separate renaturation plots are superimposed for comparison. (a): (L1 + L2)-larval DNA ( $\Delta$ — $\Delta$ ) with tracer amount of  $^{32}\text{P}$ -adult DNA ( $\blacktriangle$ — $\blacktriangle$ ). (b): Adult DNA ( $\circ$ — $\circ$ ) with tracer amount of  $^{32}\text{P}$ -adult DNA ( $\bullet$ — $\bullet$ ). In each case,  $C_0t$ 's are calculated from the total DNA.

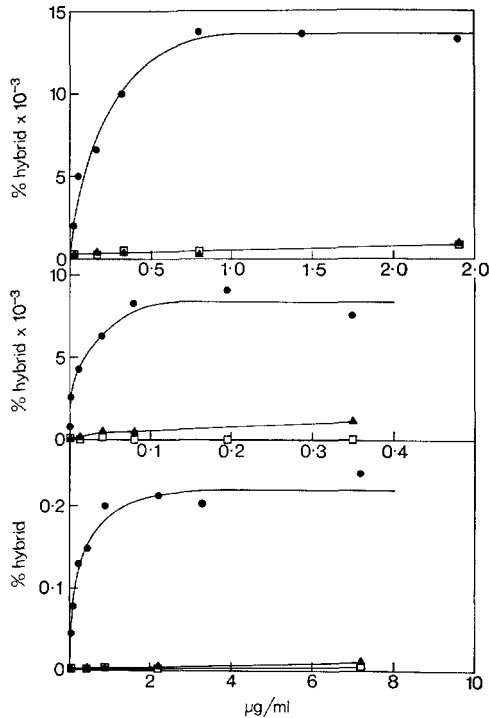


FIGURE 5.—Hybridization of  $^{32}\text{P}$ -RNA's (specific activity 82 C/M) with filter-bound DNA's (8  $\mu\text{g}$  per filter).  $\bullet$ — $\bullet$ : *C. elegans* DNA;  $\square$ — $\square$ : *E. coli* DNA;  $\blacktriangle$ — $\blacktriangle$ : blank filter.

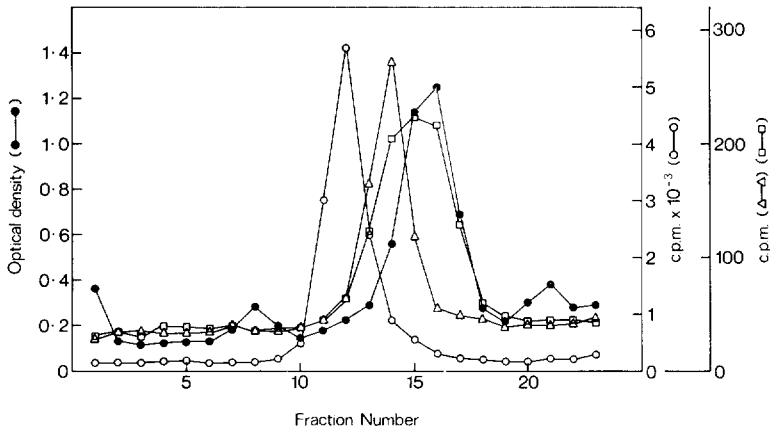


FIGURE 6.—Hybridization of  $^{32}\text{P}$ -RNA's (specific activity 68 C/M) with DNA fractions from a caesium chloride density gradient. ●—●: optical density after dilution; □—□: 4S RNA bound; △—△: 5S RNA bound; ○—○: (28 + 18)S RNA bound. The optical density peak in fraction 8 is due to a marker of *P. aeruginosa* DNA.

fractions were slightly contaminated with fragments from (28 + 18)S RNA and hybridizations were therefore carried out in the presence of excess non-radioactive high molecular weight ribosomal RNA. This was purified by sucrose gradient centrifugation from ribosomes isolated by conventional methods. The results are shown in Figure 5 and summarized in Table 1. The distribution of the sequences corresponding to these RNA's with respect to the density of the DNA was measured by RNA-DNA hybridizations with fractions from a caesium chloride equilibrium centrifugation (Figure 6). The three groups of sequences are not clustered, but the sharp 5S peak obtained suggests that the 5S sequences are clustered. As already mentioned, the ribosomal RNA's hybridize with a fraction corresponding to the dense satellite.

#### DISCUSSION

There is good agreement between the chemical estimation of the haploid DNA content and the value derived from the study of renaturation. This may be taken as evidence that the unit genome (LAIRD 1971) in *C. elegans* is contained in the haploid set of chromatids and that the slowly renaturing sequences are represented uniquely in this genome. Our results are very similar to those found in *Drosophila* by LAIRD (1971). In both organisms, most of the DNA is unique and repetitious DNA is only a relatively small fraction. *Drosophila* contains about 70% more unique DNA than *C. elegans*; whether this reflects a difference in the number of genes or the size of the genetic units is not known. The haploid content of DNA in *C. elegans* of  $0.8 \times 10^8$  base pairs is only 20 times that of *E. coli* and is the smallest value for an animal. SYN and PASTERNAK (1970) measured a haploid DNA content of  $0.426 \times 10^{-12}$  g in another small nematode, *Panagrellus*



*silusiae*, by cytophotometric methods. This corresponds to about 100 times the DNA of *E. coli*, which is much larger than the value for *C. elegans*, but a study of renaturation was not carried out. TOBLER, SMITH and URSPRUNG (1972) measured the rate of annealing of DNA from cells of *Ascaris lumbricoides*; from their curves one may estimate a DNA content about 100 times that of *E. coli*.

The somatic cells of *Ascaris* lack about 30% of the DNA sequences which are present in germ cells (TOBLER and URSPRUNG 1972). This is the result of the chromatin diminution that occurs during development. It is of interest to know whether this is a general feature of nematodes.

No evidence for diminution could be found in *Panagrellus* by SIN and PASTERNAK (1970). We tried to detect this in *C. elegans* by comparing larval and adult DNA. A young larva contains only four primordial germ cells while an adult may contain a thousand or more gametes; thus less than 1% of larval DNA and more than 5% of adult DNA is germ line. We found no differences between the two DNA's, but our test is comparatively insensitive, and we cannot eliminate the possibility of chromatin diminution. However, it is unlikely that there is chromosome elimination in *C. elegans* because, as shown by BRENNER (1974), the number of linkage groups is the same as the number of chromosomes determined in germ cells by NIGON (1949), and all the linkage groups contain several genes which affect somatic tissues.

We are indebted to Dr. D. L. BAILLIE for isolating the <sup>32</sup>P-labelled RNA species.

#### LITERATURE CITED

- BEHME, R. and J. PASTERNAK, 1969 DNA base composition of some free-living nematode species. *Can. J. Genet. Cytol.* **11**: 993-1000.
- BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71-74.
- BRITTEN, R. J. and J. SMITH, 1969 In: *Yrbk. Carnegie Inst. Washington* **68**: 378-386.
- BURTON, K., 1968 Determination of DNA concentration with diphenylamine. In: *Methods in Enzymology*. Edited by GROSSMAN and MOLDAVE. **12B**: 163-166.
- GILLESPIE, D. and S. SPIEGELMAN, 1965 A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* **12**: 829-842.
- KOHNE, D. E. and R. J. BRITTEN, 1971 Hydroxyapatite techniques for nucleic acid reassociation. In *Procedures in Nucleic Acid Research*. Edited by CANTONI and DAVIES **2**: 500-512.
- LAEMMLI, U. K., 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.
- LAIRD, C. D., 1971 Chromatid structure: relationship between DNA content and nucleotide sequence diversity. *Chromosoma* **32**: 378-406.
- MARMUR, J., 1961 A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**: 208-218.
- NIGON, V., 1949 Les modalités de la reproduction et le déterminisme de sexe chez quelques Nématodes libres. *Ann. Sci. Nat., Zool., ser. 11*, **11**: 1-132.
- SCHILDKRAUT, C. L., J. MARMUR and P. DOTY, 1962 Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**: 430-443.

- SIN, W. C. and J. PASTERNAK, 1970 Number and DNA content of nuclei in the free-living nematode *Panagrellus silusiae* at each stage during postembryonic development. *Chromosoma* **32**: 191-204.
- STUDIER, F. W., 1965 Sedimentation studies of the size and shape of DNA. *J. Mol. Biol.* **11**: 373-390.
- TOBLER, H., K. D. SMITH and H. URSPRUNG, 1972 Molecular aspects of chromatin elimination in *Ascaris lumbricoides*. *Devel. Biol.* **27**: 190-203.
- WETMUR, J. G. and N. DAVIDSON, 1968 Kinetics of renaturation of DNA. *J. Mol. Biol.* **31**: 349-370.

Corresponding editor: A. CHOVNICK