One Strand Equivalent of the *Escherichia coli* Genome Is Transcribed: Complexity and Abundance Classes of mRNA

Abstract. *DNA-RNA hybridization experiments show that essentially all of the genomic information is transcribed. High, intermediate, and rare abundance classes of messenger RNA (mRNA) are present, and their estimated complexities are equal to about 240, 1300, and 700 average-sized mRNA species, respectively. The high abundance mRNA species are present, on average, two to three copies per cell and constitute about 95 percent of the mRNA mass. Intermediate abundance mRNA species are present, on average, about once per 35 cells. The relative abundance and complexity of these mRNA classes correspond well with previous respective measurements on protein. Rare RNA species are thought to represent maximally repressed genes. Analysis of RNA synthesized in vitro by isolated nucleoids (chromosomes) suggests that sense and nonsense sequences are extensively interspersed on a given strand of the DNA.*

More than 650 loci in the chromosome of *Escherichia coli* K12 have been assigned map locations (*1*), but the *E. coli* genome contains sufficient information, if we assume asymmetric transcription, to code for about 2300 messenger RNA (mRNA) species, 1700 nucleotides in length. The extent to which the genome is transcribed has been estimated from RNA-DNA hybridization studies to be about 20 to 30 percent (*2, 3*). In these prior studies very infrequent transcripts were not measured, and thus the question arises whether the remaining 70 to 80 percent of the genome also codes for RNA or has other functions.

We now present experiments in which infrequent RNA species were measured by RNA-driven hybridization reactions taken to high C_0t (C_0t is the concentration of nucleotides in moles per liter times the time in seconds). Our results show that nearly all of one strand equivalent of the *E. coli* genome is transcribed. The kinetics of the hybridization of DNA, in an RNA-driven reaction, show that three general abundance classes of putative mRNA are present. The complexity and relative mass of the high and intermediate abundance classes of mRNA correspond well with respective measurements on proteins analyzed previously by two-dimensional electrophoresis (*4*). In addition we provide evidence, using RNA synthesized in vitro by isolated nucleoids (*E. coli* chromosomes), that nonsense sequences in a given strand of DNA are frequently adjacent to sense regions.

The total RNA was isolated from *E. coli* growing exponentially in minimal media. Previous studies have shown that mRNA is 2 to 3 percent of the total RNA in *E. coli* grown under similar conditions (*5, 6*). In our hybridization studies, the C_0t has been calculated on the assumption that 2.5 percent of the total RNA mass contains essentially all of the sequence complexity. The bulk RNA in *E. coli*—ribosomal RNA (rRNA) and transfer RNA (tRNA)—is complementary to about 0.5 percent of the DNA (*7*); thus complex RNA is referred to henceforth as mRNA.

RNA-driven DNA-RNA hybridization is shown in Fig. 1A. Since both reactants, ^{3}H-labeled DNA and RNA, are in solution, some DNA renaturation occurred. Renaturation, which was slight because very low concentrations of ^{3}H-labeled DNA were used, was corrected for by three different methods; each yielded similar values. The portion of the total input ^{3}H-labeled DNA that renatured ranged from 0.5 percent at lowest C_0t to 6.5 percent at highest C_0t. The low C_0t value probably represents zero-order renaturation of "hairpin" DNA (*8*). The hybridization curve shows that about 48 percent of the total DNA (96 percent of the genomic complexity on the assumption that transcription is asymmetric) is complementary to the RNA. Inflections in the hybridization curve occur between C_0t 0.5 to 1 and C_0t 50 to 100. Thus three general abundance classes of mRNA appear to be present; these hybridize largely between C_0t 0 to 1, 1 to 100, and 100 to 2000, respectively. The end of the first transition is indicated by an inflection between C_0t 0.5 and 1.0 (inset in Fig. 1A). This transition is not sharply delineated probably because there are gradations in mRNA copy frequencies between classes. To confirm the presence of a high abundance class, DNA-RNA hybridization was stopped at C_0t 2. Hybridized ^{3}H-labeled DNA (about 5 percent of the total input DNA) was isolated by the low-salt ribonuclease method (*9*), and again hybridized with total RNA (Fig. 1C). As shown, this DNA fraction hybridized very rapidly compared to total ^{3}H-labeled DNA. In comparison, renaturation of *E. coli* DNA is much slower, as is expected in a reaction driven by a complex population of unique sequences. Thus, it is evident that there is a class in the total mRNA to which about 5 percent of the DNA rapidly hybridizes. With ^{3}H-labeled DNA isolated at C_0t 2, transitions indicating heterogeneity in copy frequency are more evident in the high abundance class mRNA than when total ^{3}H-labeled DNA was used. About 10 percent of the ^{3}H-labeled DNA isolated at C_0t 2 would be expected to be complementary to rRNA and tRNA. The very rapid component shown in Fig. 1C probably represents, in part, the hybridization of this DNA.

The estimated complexities and rate constants for hybridization of the three general abundance classes of mRNA are given in Table 1. The fraction of the total mRNA mass contained in each of the general abundance classes was estimated from the ratio of the observed rate constant to that of the rate constant expected if all the RNA was contained in the given abundance class (*9*). For example, since the high abundance class rapidly saturates complementary ^{3}H-labeled DNA, it obviously constitutes a large portion of the mass of the mRNA. This is borne out by the ratio of the observed to the expected rate constant for this mRNA class (Table 1). Rate constants (K) for the renaturation or hybridization of nucleic acids are inversely pro-

Table 1. Abundance classes of mRNA in *E. coli*. Genomic complexity (percent) is estimated from the hybridization curve (Fig. 1A), and transcription is assumed to be asymmetric. The expected rate constant is that predicted if the given class of RNA comprised all of the mRNA. The observed K was determined from the C_0t required for half reaction ($C_0t_{1/2}$) of a class by rearrangement of the expression: $C_0t_{1/2} = \ln 2/K$. The percentage of the total mRNA mass was calculated as: $(K_{obs}/K_{exp}) \times 100$. The number of different mRNA species is expressed in units of 1.7 kilobases (*11*). The copies per cell were estimated by dividing the mass of mRNA per cell (daltons) of a given class by the number of species times the number of daltons per molecule.

mRNA abundance class	Genome complexity (%)	$K_{expected}$ (mole^{-1} sec^{-1})	$K_{observed}$ (mole^{-1} sec^{-1})	Fraction of mRNA mass (%)	Estimated number species	Copies per cell (No.)*
High	10	3.4	3.1	~ 91–96	240	2–3
Intermediate	56	0.6	0.025	~ 4–5	1300	0.03
Rare	30	1.1	0.001	~ < 1	700	0.001–0.003

*Average.

portional to the sequence complexity. If the rate constant of *E. coli* DNA renaturation is taken as a standard [for rationale, see (9)], the expected rate constant of a reaction driven by an RNA population of known complexity, which comprises all of the RNA present and in which the copy frequency of each of the species is similar, was calculated as:

$$K = \frac{2 \times C_E \times 0.3}{C_R} \times \frac{450}{800}$$

where 2 is a correction for an RNA driven reaction. In RNA-driven reactions, the rate constant is twice that of the reassociation of DNA of equal sequence complexity since, per unit mass, a given sequence is present twice as often, if we assume asymmetric transcription. The value 0.30 is the rate constant per mole per second for the renaturation of *E. coli* ^{3}H-labeled DNA fragments used in our experiments. Renaturation is shown in Fig. 1B. The complexity standard, C_E, is the *E. coli* genome, or 4.2×10^6 nucleotide pairs; C_R is the complexity in nucleotides of an RNA class under consideration, which is, for example, 4.1×10^5 for the high abundance class complementary to about 5 percent of the DNA. The correction factor (450/800) is applied in the case where the length of the driver (RNA) is greater than that of the ^{3}H-labeled DNA tracer (*10*).

The hybridization curve is internally consistent with the mass values given in Table 1. For example, the high abundance class was estimated to be 91 percent of the mRNA mass on the basis of the ratio of the observed to the expected rate constant. A similar value is obtained indirectly by consideration of the ratio of observed to the expected K for the intermediate abundance class. The expected K for this class is 0.6 mole^{-1} sec^{-1}, but the observed was 0.025 mole^{-1} sec^{-1}. Thus, the reaction proceeds at a rate 24 times slower than would be expected if all of the mRNA present was contained in this class. The intermediate class therefore is about 4 percent of the mRNA mass. Since the rare class is a negligible portion of the mRNA mass, the abundant class appears to be, by this indirect estimate, 96 percent of the mRNA mass, a value similar to that obtained by direct consideration of the observed K. These estimations are consistent with Kennell's observation that 10 percent of the DNA of *E. coli* is complementary to essentially all (more than 99 percent) of the mass of the mRNA that had been labeled for either a short or long period (*2*).

The number of different mRNA sequences may be only roughly estimated from saturation hybridization data since the length of the various mRNA sequences is imprecisely known. However, we have used mapping data of the *E. coli* chromosome to estimate the size of an average gene. From the most intensely mapped region in the *E. coli* chromosome (72 map minutes), we calculate that the average "gene" in this region is 1700 nucleotides in length (*11*). On the basis of 1700 nucleotides as the length of an average mRNA sequence, the number of different mRNA's represented in the various abundance classes was estimated from observed complexities (Table 1).

From complexity values and mass estimates, we have also estimated the average number of copies per cell of each mRNA species for each abundance class (Table 1). In these estimations, the total

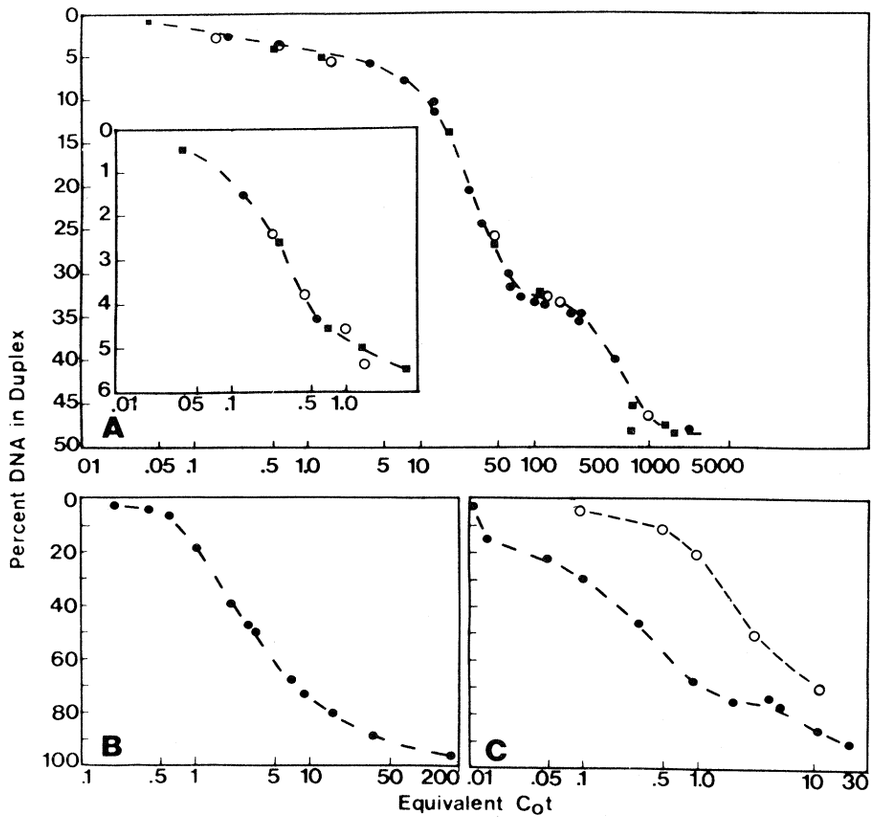

Fig. 1. (A) RNA-driven hybridization of ^{3}H-labeled DNA. The equivalent C_0t is the C_0t normalized to 0.18M Na^+. The amount of hybridized DNA was determined by hydroxylapatite (HAP) chromatography. C_0t is plotted on the basis that 2.5 percent of the total RNA drives the reaction. The values shown were corrected for DNA-DNA renaturation by three different methods. (■) Samples were equally divided, and in one portion total duplex was measured by HAP chromatography, and the other portion was treated with ribonuclease in low salt at 39°C prior to HAP chromatography. The ribonuclease-resistant fraction was considered as DNA-DNA and was subtracted from the total duplex (*9*). (•) Values corrected relative to reactions in which polyuridylic acid [poly(U)] was substituted for RNA. (○) Hybrid values obtained after treatment with S1 nuclease specific for the single strand (*21*) and corrected relative to poly(U) controls. The inset indicates an experiment showing in more detail the hybridization reaction at low C_0t. (B) Renaturation of ^{3}H-labeled DNA used as tracer in the hybridization experiments. The reaction was driven with sheared, unlabeled *E. coli* DNA of the same fragment size. (C) (•) Repeated hybridization of the ^{3}H-labeled DNA obtained as hybrid by C_0t (about 5 percent of the total DNA) and purified by the low salt–ribonuclease procedure (*9*). (○) Renaturation of total ^{3}H-labeled DNA driven by unlabeled *E. coli* DNA. The strain *E. coli* D10 was grown in minimal media with glycerin and supplemented with methionine (*22*). Cells were lysed with lysozyme and sodium dodecyl sulfate, the DNA was sheared, and RNA was extracted by a hot phenol-chloroform procedure (*23*). The RNA was further purified by treatment with deoxyribonuclease and Pronase and subsequent passage through Sephadex G-100. ^{3}H-Labeled DNA was purified from isolated nucleoids (*22*) by phenol extraction and equilibrium centrifugation in CsCl. DNA was sheared to 400 to 500 nucleotides by passage through a pressure cell equipped with a ball valve. Specific activity of the DNA was 3×10^6 count/min per microgram. For hybridization, RNA was dissolved (0.05 to 12 μg/μl) in 0.12 to 0.4M sodium phosphate buffer containing 0.05 percent sodium dodecyl sulfate and 0.001M EDTA. Heat-denatured DNA was added such that 3000 to 6000 count/min were contained in individual samples placed in capillary tubes. Reaction mixtures were incubated at 67°C. Samples were removed at various values C_0t and radioactivity in duplex and single-strand fractions obtained by HAP chromatography were measured by liquid scintillation counting. The temperature at which half of the hybridized DNA was released as single strands (T_m) was 89° to 90°C in 0.11M sodium phosphate buffer, as determined by thermal elution from HAP as specified by Martinson (*24*).

RNA per *E. coli* cell was taken as 1.45×10^{10} daltons, of which about 3.6×10^8 daltons (2.5 percent) is mRNA (*5*). For example, the high abundance class mRNA is about 95 percent of the mass of total mRNA, or about 3.4×10^8 daltons per cell. Since the diversity of this class is equal to 240 molecules 1700 nucleotides in length or 1.3×10^8 daltons, the average number of each species is $(3.4 \times 10^8)/(1.3 \times 10^8)$ or about 2.5 copies per cell. Heterogeneity in copy frequency exists (Fig. 1C), so that some mRNA's in the general classes may be present to lesser or greater extent than the average. For example, in a tryptophan (Trp) constitutive mutant of *E. coli*, 25 copies of Trp-mRNA molecules per cell may be present (*12*). Ribosomal protein mRNA's would also be expected to be among the most abundant species. However, the C_0t range of each of the abundance classes (about two orders of magnitude) suggests an absence of extensive heterogeneity in copy frequency among most of the different species within the classes (*13*).

Our estimation, that each of the mRNA species in the intermediate class is present, on average, at about 1 copy per 35 cells, may seem to be below the level required physiologically. However, mRNA at this concentration is adequate to maintain many of the enzymes and other proteins in amounts that are necessary for normal function (*14*).

We estimate from the observed rate constant that the frequency of the rare mRNA is about 1 copy of each species per 1000 cells. However, this may be an underestimate because in "high C_0t" reaction mixtures the total RNA was 10 to 12 μg/μl, whereas 0.2 to 3.0 μg/μl was used in the intermediate C_0t range. The greater viscosity may have slowed the reaction somewhat. RNA before incubation at 60°C, at a concentration of 10 μg/μl, had a viscosity about equal to 40 percent sucrose at the same temperature, as determined by the falling sphere method. The rate constant for DNA renaturation has been found to be more than threefold less in the presence of 40 percent sucrose than in no sucrose (*15*). Therefore the $C_0t_{1/2}$ of the rare class may be, in comparison to the abundant and intermediate classes, two to three times less than observed if a viscosity correction is made. Even if this correction is made, the copy frequency is still low, being about 1 copy per 300 cells.

O'Farrell has determined the relative abundance and the diversity of proteins in *E. coli* by two-dimensional electrophoresis (*4*). About 1200 different proteins, excluding basic proteins, have been detected by this method. The abundant class (protein species that individually made up 10 to 0.05 percent of the total labeled protein) contained about 230 different species. This value is close to the 240 species that we estimate are present in the most abundant mRNA class. Relative comparisons of mass of the abundant protein and mRNA classes are also in close agreement. The abundant class proteins observed by O'Farrell constituted 95 percent (or more) of the mass of labeled protein. In accord with this, we estimate that the abundant class mRNA also constitutes about 95 percent of the mass of the mRNA.

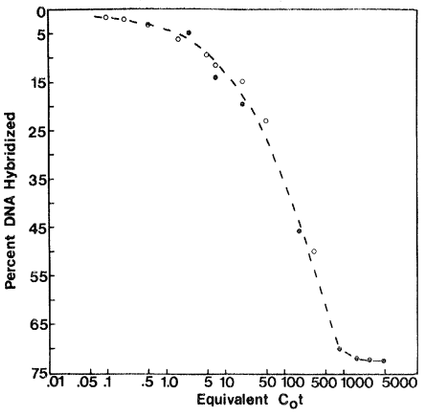

Fig. 2. Hybridization of RNA from *E. coli* nucleoids. RNA from two preparations (○, ●) was hybridized to ^{3}H-labeled DNA as described (Fig. 1). Correction for DNA-DNA was by the low salt–ribonuclease method. The C_0t was calculated on the basis that mRNA comprised 15 percent of the total RNA (*25*). Nucleoids were prepared from lysed cells as described (*26*). They were incubated for 30 minutes at 37°C in a medium containing 1*M* NaCl, 1 m*M* of required nucleoside triphosphates, and 10 m*M* $MgCl_2$. RNA was prepared as described (Fig. 1).

We equate protein species present at an abundance of 0.01 percent or less of the total protein as representing the intermediate abundance class mRNA's. O'Farrell observed about 900 proteins that are in this general abundance range (*4*). Since we estimate the complexity of the intermediate class of mRNA to be about 1300 species, there may be some discrepancy between the complexities of the mRNA and protein. Some protein species in this abundance class may not have been detected. The discrepancy is further reduced by the addition of basic proteins whose diversity could easily be 100 to 200 species. Also, our estimate of the average size of functional mRNA may be lower than is actually the case. It is also possible that RNA species other than mRNA constitute some portion of this abundance class. In view of these considerations there is no obvious discrepancy between the diversities of mRNA and protein. The relative mass of these respective protein and mRNA classes is also similar since both make up about 5 percent of their respective total masses. These complexity and abundance comparisons further suggest that the abundance of many proteins is determined by the amount of respective mRNA. These comparisons also support the assumption that RNA which drives the hybridization reaction is in fact mostly mRNA.

The translational products of rare RNA sequences (if messenger, and if translated) would not have been detected since each of these protein species would constitute considerably less than 10^{-5} percent of the total protein, thus being below resolution by autoradiography used by O'Farrell (*4*). Since a class of proteins representative of this RNA has not been identified, the function of the DNA coding for this RNA is open to question. Genes that are maximally repressed could be represented by mRNA sequences in the rare copy class. For example, when the lac operon is maximally repressed, some lac-mRNA is present, but there is less than one ribosome which bears β-D-galactosidase per 100 cells (*16*). Thus an estimate of the upper limit of lac-mRNA concentration would be in the range of 1 copy per 100 to 300 cells. Therefore, mRNA representative of repressed genes may constitute some or all of the rare copy RNA species [see (*2*) for discussion of this point]. It is also possible that some of the RNA in this class is a transcript from nongenetic sequences. Some portion of the DNA may lack code or regulatory function; occasional transcription of this DNA may permit operation of certain processes of selection.

We have also examined the hybridization of RNA from isolated bacterial chromosomes (nucleoids) that were transcribed in vitro (Fig. 2). Purified nucleoids retain their endogenous RNA polymerase molecules with their associated nascent RNA chains, and these are apparently distributed on the operons as they were in vivo (*17*). When nucleoside triphosphates and Mg^{2+} are added, the in vitro RNA synthesized in the presence of 1.0*M* NaCl occurs exclusively by elongation of the preexistent nascent chains (*17, 18*). Termination factors are dissociated from the nucleoid during purification so that termination sites requiring a factor (like rho) may be overrun. The newly synthesized RNA chains attain lengths of 2000 to 10,000 nucleotides. At about C_0t 2, from 5 to 6 per-

cent of the DNA is hybridized (Fig. 2), as was the case for RNA made in vivo (Fig. 1A). This result may be interpreted in two ways. (i) Failure to observe an increase in complexity of high abundance class mRNA may indicate that termination signals are obeyed on the frequently transcribed operons. This could happen in the absence of regulatory proteins as in the case with transcription in vitro of the rRNA genes (*18*). (ii) Polymerase molecules on the frequently transcribed genes do overrun the termination signals in vitro, but operons that are infrequently transcribed in the cell are not extensively interspersed with the frequently transcribed operons. In this case, if they were highly interspersed, transcripts from many of the less active sequences would have been increased in abundance and a more complex high abundance class would have been observed. Interspersion involving more than 6000 nucleotides cannot be ruled out since this is the maximal length of most of the in vitro transcripts.

Saturation values obtained with the nucleoid RNA show that about 73 percent of the total DNA was transcribed (Fig. 2). This means that during in vitro synthesis transcription is not exclusively asymmetric. Since the endogenous RNA polymerases did not usually transcribe beyond about 6000 nucleotides, an increase in complexity equal to 25 percent of the DNA suggests that there are frequent interspersions between sense and nonsense sequences on a given strand of DNA. Examples of such interspersions have been found for prokaryotic chromosomes (*1*, *19*). This interpretation depends on earlier findings that RNA chains are not initiated under the conditions of in vitro synthesis (*17*). The greater complexity of the nucleoid RNA is observed largely at high C_0t (100 to 2000 mole · sec/liter). Thus, this sequence interspersion may be concentrated in infrequently transcribed regions of the DNA. However, if termination signals are only very rarely missed in vitro, transcripts of nonsense sequences adjacent to frequently transcribed operons would also be observed at high C_0t.

The inflection of the curve at C_0t 100 (Fig. 1A) is not evident in Fig. 2. Lack of data on nucleoid RNA leaves unclear the question of whether this transition has been eliminated by increased gradation of copy frequency of the in vitro synthesized RNA.

In conclusion, we have observed maximal hybridization values of about 48 percent in various experiments with three preparations of RNA. Considering that certain regions of promotor and terminator sequences are not transcribed [for example, 1 to 2 percent of the lac operon is not transcribed (*20*)], a saturation value of 48 percent indicates that all of the genomic information is transcribed, if transcription is asymmetric.

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11. The region at minute 72 on the *E. coli* genetic map contains 24 known loci (*1*). Since 4.1×10^4 base pairs of DNA are transferred per minute, the average gene in this region of the chromosomes is $(4.1 \times 10^4)/24$ or 1.7×10^3 nucleotides in length. This could be an overestimate of average size if all the loci located at 72 minutes have not been identified, or if this region contains genes that are larger than average. An underestimate would result if the genes in this area are smaller than average, or if the same gene has been characterized phenotypically such that two or more loci actually represent the same gene. However, this approach may provide a better estimate than would the use of the average polypeptide length because such an average is largely determined by abundant class proteins, which may not be representative of the diverse, infrequent proteins. Furthermore, mRNA would be somewhat larger than reflected by respective proteins if untranslated sequences exist.
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Kepone-Induced Scoliosis and Its Histological Consequences in Fish

Abstract. *Scoliosis in fish is caused by several diverse agents that possibly act on the central nervous system, neuromuscular junctions, or ionic metabolism. The organochlorine pesticide Kepone induces scoliosis in the sheepshead minnow. Some effects associated with Kepone-induced scoliosis in these fish are disruption of myotomal patterns, inter- and intramuscular hemorrhage, fractured centra of vertebrae, and death. The histological syndrome of Kepone poisoning in fish and the clinical syndrome in humans suggest that the nervous system is a primary target for Kepone and that scoliosis is a secondary effect of Kepone poisoning in fish.*

We have found that exposure of sheepshead minnows (*Cyprinodon variegatus*) to a relatively low concentration of the organochlorine Kepone (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*c*,*d*]pentalen-2-one) produces a syndrome in the fish in which scoliosis, resulting in severe spinal column injury, is one cardinal sign. Scoliosis, lateral curvature of the spine, has been reported to occur in several species of fish as a result of dietary deficiencies (*1*), organophosphate and carbamate poisoning (*2*), heavy metal exposure (*3*), and parasitic infections (*4*). This report is concerned with the severe histological effects of scoliosis in sheepshead minnows exposed to Kepone in the laboratory.

Hansen and co-workers (*5*) first observed scoliosis and blacktail (loss of neurologic control of melanocytes in the caudal region) as a syndrome of Kepone exposure in sheepshead minnows. They experimentally demonstrated that induction of scoliosis with Kepone was a dose-dependent, time-related phenomenon.