Macromolecular Complexes Produced by 1,3-Propanesultone

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SUMMARY

1,3-Propanesultone produces in vitro complexes between DNA and DNA, DNA and RNA, RNA and RNA, DNA and proteins, and possibly RNA and proteins. All these interactions were detected with a variety of independent analytical techniques. Increased attachment of the 1,3-propanesultone-treated DNA to the microsomal membrane and enhanced adsorption to and penetration into Ehrlich ascites tumor cells were also observed. 1,3-Propanesultone was the second small-ring alkylating carcinogen studied in this laboratory that was found to produce such macromolecular complexes. The possibility that these highly diverse structures may be involved in chemical mutagenesis and tumorigenesis is considered.

INTRODUCTION

We have recently reported that a monoalkylating carcinogen (BPL) produces severe alterations in DNA that lead to increased binding of the altered DNA to cellular membranes (17) and purified proteins (27) as well as to other macromolecules including other DNA chains (24). Further experiments (unpublished) revealed that in the presence of BPL similar complexes are also formed by RNA. The techniques applied in these studies included: centrifugation in sucrose (velocity) and cesium chloride and cesium sulfate (isopycnic) density gradients; chromatography on MAK gels; electrophoresis in polyacrylamide-agarose gels; membrane filtration; and electron microscopy. All these interactions were detected with a variety of independent analytical techniques. Increased attachment of the 1,3-propanesultone-treated DNA to the microsomal membrane and enhanced adsorption to and penetration into Ehrlich ascites tumor cells were also observed. 1,3-Propanesultone was the second small-ring alkylating carcinogen studied in this laboratory that was found to produce such macromolecular complexes. The possibility that these highly diverse structures may be involved in chemical mutagenesis and tumorigenesis is considered.

This stage the nucleic acids were seen to attach firmly to the walls of centrifuge tubes and even to nitrocellulose membranes during filtration. Under the electron microscope DNA progressively assumed the form of a highly complex network of interconnected, branched “megamolecules,” suggesting that the ends of broken DNA fragments associated with other DNA chains (24). We suggested (24, 27) that such macromolecular interactions may play an important role in chemical mutagenesis and carcinogenesis since the great variability of such complexes will cause some of them to escape “normal” repair mechanisms and may, in consequence, lead to cell death or to profound alterations in cellular phenotype and genetic makeup.

With this possibility in mind, we decided to evaluate the potential of several carcinogenic chemicals to induce such macromolecular interactions both in vitro and in vivo. Cross-linking by polyalkylating agents of DNA to protein (6, 30) and of the 2 DNA strands (5, 15, 25) has been known for some time. It was generally assumed that monoalkylating compounds should not cause cross-linking. Recently, however, observations from this (17, 24, 27) and from other laboratories (9) indicate that BPL in vitro and methyl methanesulfonate, another monoalkylating agent used in vivo, are capable of producing bonds between cellular macromolecules. This report summarizes our observations on the in vitro effects of another monoalkylating compound related to BPL, PS. This compound is commonly used in production of drugs, in detergents, and in food, textile, and rubber industries (31). It was found to induce tumors in experimental animals (3, 29, 31, 32) and mutations in plants (12) and coliphage T4 (2). It reacts with positions N-7 in guanosine and N-3 in adenosine and markedly decreases the melting temperature of DNA (7). Several striking similarities in the actions of BPL and PS on cellular macromolecules were discovered.

MATERIALS AND METHODS

Most of the materials and techniques used in this work were described elsewhere (17, 23, 24, 27).

Materials. Nucleic acids labeled (8) with 32P were extracted (21, 22) from bacteria (Escherichia coli Q13, Sarcina lutea, and Cytophaga johnsonii; for further information on these strains see Refs. 18 and 22) and from Ehrlich ascites cells incubated with the radioactive isotope under conditions of constant pH and temperature (14). The nucleic acids were then further purified by alcohol precipitation and gel filtration through Sephadex (Pharmacia,
The samples were then chilled on ice and diluted with buffer. The membrane was incubated for various lengths of time in discontinuous citrate-sucrose density gradients, was generally given to us by Dr. C. B. Kasper from this University.

Sarkosyl was purchased from Geigy Industrial Chemicals, Ardsley, N. Y. Bovine serum albumin, Fraction V, was purchased from Armour Pharmaceutical Company, Kankakee, Ill.; DNase I was from Worthington Biochemical Corporation, Freehold, N. J. All other chemicals used were of analytical grade.

Methods. Velocity sedimentation experiments in sucrose density gradients and measurements of the amounts of radioactive nucleic acids adsorbed to the centrifuge tube walls and bottoms were described earlier (24, 27). Electrophoresis of DNA was carried out in composite acrylamide-agarose gels according to the method of Peacock and Dingman (28). From our previous experience with DNA exposed to BPL, we know that the DNA megamolecules often do not enter the gels and are easily lost during subsequent handling prior to slicing. To prevent such losses, the gels were covered with a thin layer (1- to 2-mm) of Sephadex G-25 which effectively trapped the very-high-molecular-weight material. After the completion of the electrophoretic run, the Sephadex layer was carefully removed and the top of the gel rinsed with a small volume of distilled water. Both the Sephadex and the water rinse were then combined for subsequent counting.

Chromatography on MAK columns (26) was performed under standardized conditions of constant temperature and flow rate (13, 21). The amount of radioactive DNA retained on such columns was measured at the end of the experiment by suspending column material in water, measuring the volume of the suspension, and pipetting aliquots of various volumes on aluminum planchets. Subsequently, the radioactivity was measured in a low-background, gas-flow counter and the results extrapolated to 0 concentration of kieselguhr. In this, as well as in all other assays of \(^{32}P\) radioactivity, the results were corrected for the decay of the isotope.

Equilibrium density gradient centrifugation of DNA and DNA-protein complexes in CsCl-Sarkosyl was carried out according to the earlier description (27). The techniques used to study binding of DNA to rat liver microsomal membrane and the effects of various biologically active chemicals on this reaction were described previously (16–18, 20, 23). The membrane, purified (11) by centrifugation in discontinuous citrate-sucrose density gradients, was generously given to us by Dr. C. B. Kasper from this University. The membrane was incubated for various lengths of time with E. coli DNA, in the presence and in the absence of PS. The samples were then chilled on ice and diluted with buffer (0.01 M phosphate buffer (pH 7.2)-0.1 M NaCl); saturated CsCl was added to achieve density of about 1.2 g/ml. The samples were subsequently centrifuged until equilibrium, the centrifuge tubes were viewed to establish the position and appearance of the membrane band, and fractions were collected from the bottom. Distribution of radioactivity and the slope of the density gradient were established as previously described (18).

Binding of DNA to Ehrlich Ascites Tumor Cells. The cells were collected from tumor-bearing mice on the 7th day after injection into their peritoneal cavities. The cells were washed twice with Hanks' buffered solution (Microbiological Associates, Bethesda, Md.) and suspended in TSM buffer. Radioactive E. coli DNA was added (40 ng/10⁶ cells) and after various periods of incubation at 37° aliquots were withdrawn, diluted with TSM, washed once, and resuspended in TSM with or without DNase I, 20 µg/ml. After 10 min of additional incubation at 37°, the cells were diluted with cold TSM, centrifuged, washed once with cold TSM, and centrifuged again, and the amounts of radioactive material in the pellet were determined. In all these experiments, the cells were pelleted by centrifugation for 10 min at 1000 rpm in a HG-4L Sorvall rotor. In separate experiments, we have found that the PS-treated DNA alone did not sediment to the bottom of the centrifuge tube under these conditions.

Protein was estimated by the Folin procedure as described by Bailey (1).

RESULTS

Effects of PS on the Apparent Molecular Weight of DNA. Following exposure of E. coli \([^{32}P]\)DNA to PS (in this and subsequent experiments described in this report, the carcinogen was in 10⁴ to 10⁸ molar excess over DNA or RNA) and centrifugation in sucrose density gradients, 2 classes of radioactive material became evident (Chart 1): 1 DNA fraction which sedimented more slowly than the untreated control and was recovered in the 4 to 8S region of the gradient (Chart 1, Fractions 37 to 41); and a rapidly sedimenting DNA material observed deeper in the gradient than the control DNA. This fraction of progressively increasing molecular weight comprised the bulk of radioactive nucleic acid under our conditions. The proportion between the 2 DNA classes (i.e., the slow- and the fast-sedimenting DNA's) varied between experiments. We have noted that, in conformation with our earlier observations on BPL-treated DNA (24), this proportion was shifted in favor of the fast-sedimenting fraction if the DNA was at a higher concentration when incubated with PS. Conversely, diluted solutions of DNA produced more of the slow-sedimenting fraction in the presence of PS.

A substantial fraction of the radioactive PS-treated DNA was recovered at the bottom of the centrifuge tube after completion of the collection of samples. In experiments depicted in Chart 1A, the percentage of total DNA recovered from the bottom was distributed as follows: less than 1% in the control; 1% at 15 min; 3% at 30 min; 22% at 60 min. In experiments shown in Chart 1B, percentages found were less than 1% in the control, 4% in the tube with the sample exposed to 1% PS, and 18% at 2% concentration.
of this carcinogen (1% concentration of PS corresponds to approximately 0.11 M).

In addition, up to 5% of the total PS-treated DNA was attached to the top of the centrifuge tube in an area where the sample was originally layered. The amount of radioactivity recovered at this location was higher in experiments in which DNA was exposed to high concentrations of PS and/or for prolonged periods of time, and especially when the tubes with the applied PS-treated DNA were allowed to stand for some time at room temperature before the beginning of centrifugation.

The rapidly sedimenting DNA (megamolecules) (24) was also observed in alkaline sucrose (Chart 2), thus indicating that the linkages involved in the formation of these complexes are stronger than hydrogen bonds.

In parallel experiments, the PS-treated DNA was analyzed by electrophoresis in agarose-polyacrylamide gels. Chart 3 demonstrates the results of a typical experiment. The untreated E. coli DNA moved through the gel as a homogenous material and very few counts were recovered from the top and from below the 30-mm level of the gel. In contrast, most of the PS-treated DNA failed to enter the gel and was recovered in the Sephadex layer. Another fraction of this DNA appeared as a heterogenous material of a significantly lower molecular weight than that of the control sample. We were able to manipulate the relative proportions between the low- and the very-high-molecular-weight fractions by altering the concentration of DNA during treatment with PS; thus repeating, with another technique, our observations described previously.

Effects of various factors on the reaction between PS and DNA were investigated. Observations made with sucrose gradient centrifugation were subsequently repeated and reproduced by gel electrophoresis. The results can be summarized as follows: (a) Denaturation of DNA by heating to 100° and fast cooling had no effect on reactivity of the DNA with PS. (b) Molecular weight of DNA had an appreciable effect on the rate of reaction between DNA and PS. The increase in the sedimentation rate in sucrose was significantly retarded for DNA sheared by sonication (30 sec, 50 watts, in a Heat Model 185 sonifier) to an average size of 2 to 3 x 10^9 daltons. (c) Both S. lutea (72% guanine plus cytosine content) and C. johnsonii (34% guanine plus cytosine) DNA's reacted with PS and the degree of reaction was comparable to that of E. coli DNA (50% guanine plus cytosine). (d) Coincubation of DNA with PS at 20° instead of the customary 40° decreased the rate of reaction severalfold. Little or no change in the sedimentation rate of DNA was observed following its incubation with PS at 0° for as long as 90 min. (e) Ionic strength and the presence of divalent cations in the incubation medium made no appreciable difference. The alterations in the sedimentation rate of DNA were quite comparable after it was incubated with PS in buffers with NaCl at concentrations between 0.05 and 0.5 M, in the presence of Mg^2+ up to 0.01 M, or in the presence of 0.02 M sodium citrate. Also the presence or the absence of Mg^2+ in sucrose gradients used for subsequent analysis did not appreciably alter the sedimentation of the PS-treated DNA. Similarly, replacement of the phosphate buffer with Tris-HCl had no effect on the reaction. (f) Variations in the concentration of hydrogen ions between pH 5.8 and 7.8 had no appreciable effect on the yield of megamolecules induced in E. coli DNA by PS.

Effects of PS on Interactions between Proteins and DNA. Results of an experiment in which E. coli DNA was incubated with PS and bovine serum albumin and then centrifuged until equilibrium in CsCl plus Sarkosyl density gradients are shown in Chart 4. DNA incubated with either albumin or PS alone does not change its density in CsCl (not shown in the chart). The latter observation was subsequently confirmed by analytical centrifugation in the Spinco Model E centrifuge. When DNA was incubated with

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Chart 1. Effects of PS on DNA sedimentation in neutral sucrose. Two μg of E. coli [32P]DNA were exposed to constant concentration (1%) of PS for various lengths of time (A) and for a constant time (30 min) to various concentrations of PS (B). The incubation was carried out at 40° in 0.1 M NaCl-0.02 M phosphate buffer (pH 7.2), and 1 mM MgSO4. The samples were then layered on the top of 5 to 20% sucrose gradients containing 0.1 M NaCl-0.01 M phosphate buffer (pH 7.2) plus 0.015 M sodium citrate and centrifuged in a Spinco SW 41 rotor for 210 mm at 38,000 rpm and at 4°. Fractions were collected from the bottom. A: ○, control; O, incubation for 15 min; ▲, 30 min; ■, 60 min. B: ○, control; ▲, incubation with 1% PS; ■, 2% PS.

Chart 2. Effects of PS on DNA sedimentation in alkaline sucrose. E. coli DNA was incubated with 1% PS for various periods of time under conditions specified in the legend to Chart 1 and centrifuged in 5 to 20% alkaline sucrose gradients (24) ○, control; O, incubated for 15 min; ▲, incubated for 30 min; ■, incubated for 60 min.
as much as 50% to the mass of the complex, as judged by the position of the complex in CsCl gradient.

In another series of experiments, DNA exposed to PS was chromatographed on MAK columns. Chart 5 demonstrates that PS-treated DNA was retained on MAK columns and that this effect was related to the duration of treatment and to the concentration of the chemical. The decrease in the amounts of such DNA eluted by the gradient of sodium chloride is due to the increased affinity of the DNA toward the methyl-esterified albumin, since no retention was observed on columns built with kieselguhr alone. These experiments give additional evidence for binding of PS-alkylated DNA to proteins.

The increase in sedimentation in neutral sucrose of DNA coincubated with PS and albumin was not greater than that observed with PS alone. This was somewhat surprising since proteins markedly increase the rate of sedimentation of DNA exposed to BPL (27). However, we were able to see the formation of rapidly sedimenting complexes in DNA treated for a short time with PS in the presence of albumin and subsequently analyzed on alkaline sucrose gradients. Under conditions used in these experiments, neither albumin nor PS alone altered the sedimentation rate of the DNA.

Binding of DNA to Nitrocellulose Filters following Treatment by PS. We have previously observed (27) that BPL-treated DNA is retained on nitrocellulose filters. Previously an increased attachment of PS-treated DNA to centrifuge tubes, and especially those made of nitrocellulose, was described. We tested, therefore, the degree of retention of E. coli DNA exposed to PS on B6 nitrocellulose filters (Schleicher and Schuell, Inc., Keene, N. H.) as a function of the duration of exposure and concentration of the chemical. Chart 6A demonstrates that PS-treated DNA and albumin added at the same time, its density in CsCl changed quite dramatically. Instead of the density of 1.71 g/ml, characteristic for E. coli DNA, the new peak appeared at a density close to 1.67 g/ml. The density of protein in CsCl is 1.44 g/ml (10) and assuming that all the density shift in DNA was due to the formation of the complex with albumin, as much as 15% of albumin must have been present in this DNA fraction. Folin analysis of the complex indicated the presence of 18% of protein. Moreover, a substantial proportion of the radioactive material was found at still lower densities, distributed in the gradient all the way up to the top of the centrifuge tube. In some of these nucleoproteins albumin may have contributed as much as 50% to the mass of the complex, as judged by the position of the complex in CsCl gradient.

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![Chart 3](https://example.com/chart3.png)

Chart 3. Effects of PS on the electrophoretic mobility of DNA in gels. One μg of E. coli [32P]DNA was incubated for 30 min with 1% PS, under conditions described in the legend to Chart 1, and analyzed by gel electrophoresis in agarose-acrylamide gels. Fraction "0," quantity of radioactive DNA not admitted into the gel and recovered from Sephadex layer at the end of electrophoresis (see "Materials and Methods").

![Chart 4](https://example.com/chart4.png)

Chart 4. Equilibrium density gradient centrifugation of E. coli DNA and E. coli DNA treated with PS in the presence of albumin. ●, radioactive DNA incubated alone; △, with 1% PS and 20 mg of albumin per ml. Incubation, 60 min at 40°. Saturated solution of CsCl was added together with 0.02% Sarkosyl; samples were centrifuged for 44 hr at 35,000 rpm in a Spinco SW 56 rotor.

![Chart 5](https://example.com/chart5.png)

Chart 5. MAK column chromatography of PS-treated DNA. E. coli [32P]DNA was exposed to PS at various concentrations for a constant incubation time (60 min) (A) or for various lengths of time with constant PS concentration (0.04%) (B). Samples were then diluted 40-fold with buffer (0.1 M NaCl-0.05 M phosphate, pH 6.7) and applied onto the column. DNA was eluted with a gradient of increasing concentration of NaCl. A: ●, no PS (0.8); △, 0.4% PS (68); ■, 1% PS (96). B: ●, no PS (0.7); △, 30 min (35); ■, 60 min (66). Numbers in parentheses, percentage of total DNA applied that was retained on the column and not eluted by the NaCl gradient during experiment.
is retained on filters and that the degree of retention is related to the time of treatment and the concentration of PS. The retention seems to be due to the increased affinity of the treated DNA toward filter material rather than to the formation of large clumps that are unable to enter the small pores of the filter (0.4 μm in diameter). Two observations favor this conclusion: (a) the degree of retention was rather insensitive to the concentration of DNA during treatment, whereas the formation of large complexes is sensitive (see above); and (b) when polypropylene filters of similar pore size were used (Nucleopore Corp., Pleasantown, Calif.) not more than 10 to 15% of the PS-treated DNA was retained compared to the amount attached to the nitrocellulose filters.

**Associations of the PS-treated DNA with Rat Liver Microsomal Membrane and with Intact Cells.** Microsomal membrane from rat liver belongs to a large group of cellular membranes that bind *E. coli* and human DNA under a variety of conditions (18). This reaction is modified by the presence of several carcinogenic and cocarcinogenic chemicals (17, 20, 23) and drugs (16). BPL, which in many respects resembles PS chemically and biologically, increases the rate of binding of DNA to this membrane severalfold (17, 20). We decided, therefore, to investigate the effects of PS on the interactions between DNA and microsomal membrane. In these experiments, DNA was coincubated with the membrane and various concentrations of PS (or for various periods of time) and centrifuged in CsCl until equilibrium (Chart 7; Table 1). At relatively low concentrations of PS, microsomal membrane banded at increasing densities in the gradient. At first, 2 distinct zones were created, the upper 1 composed of fine, homogeneously dispersed particles of membrane material, superficially resembling in appearance the membrane band in untreated control samples. A 2nd, granular zone of clumped membrane material was seen below the 1st layer. Most of the DNA was subsequently found associated with the granular zone. Further increase in the concentration of PS increased the density of the membrane band until 1 sharp, well-defined zone was seen at much higher densities than in the control. The amounts of DNA recovered with the membrane band were considerably increased over the control sample. At still higher concentrations of PS, however, a reverse process was observed; the density of the membrane decreased dramatically, in parallel to the decrease in the amounts of DNA associated with it.

The increased binding of DNA to microsomal membrane at low concentrations of PS suggested to us that exposure to this chemical may change the pattern of interaction of DNA

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**Table 1**

<table>
<thead>
<tr>
<th>Concentration of PS (%)</th>
<th>Incubation time at 40° (min)</th>
<th>Density of the membrane band (g/ml)</th>
<th>Relative amounts of [32P]DNA associated with the membrane band</th>
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<tr>
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<td>60</td>
<td>1.180</td>
<td>100</td>
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<td>30</td>
<td>1.180</td>
<td>98</td>
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<td>0.3</td>
<td>30</td>
<td>1.195a</td>
<td>188</td>
</tr>
</tbody>
</table>

*a* An additional band was visible at the density of 1.180 to 1.185 g/ml. It was subsequently determined that very little or none of the [32P]DNA associated with this material.
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with plasma membranes of intact cells. As a test of this possibility, *E. coli* DNA was coincubated with Ehrlich ascites tumor cells in the presence and absence of PS (Table 2). Untreated DNA binds appreciably to Ehrlich cells and, by 60 min of incubation at 37° up to 2% of the total DNA was found in the cellular pellet and was not removed by repeated washing with a buffered 0.9% NaCl solution. Most of this DNA was insensitive to DNase at the end of the 60-min incubation period, presumably indicating its uptake into the receptor cells. The presence of PS significantly enhanced the adsorption of DNA and most of the attached DNA was insensitive to DNase, although this insensitivity may have been caused in part by the general increase in refractivity of the PS-treated DNA toward this enzyme (unpublished). The increased adsorption of DNA was related to the concentration of PS and to the time of exposure to this carcinogen. Preincubation of DNA with PS before addition of cells further enhanced the binding and (presumably) penetration. No further increase was noted when the cells were preincubated with the chemical prior to the addition of radioactive DNA.

**Effects of PS on RNA.** Increased sedimentation rate of 32P-labeled *E. coli* or Ehrlich ascites total cellular RNA's was observed after treatment with PS. Similarly, the PS-treated *E. coli* RNA was retained on MAK columns (Chart 8) and this effect was proportional to the concentration of PS, time of exposure (not shown), and the molecular weight of RNA: the larger rRNA was more sensitive than the smaller one, while the tRNA peak was changed relatively little even at quite high concentrations of PS. Addition of unlabeled *E. coli* DNA of high molecular weight (average molecular weight higher than 10^6 daltons) enhanced the effect of PS, presumably indicating the formation of complexes between RNA and DNA in addition to those formed between various RNA's and possibly between RNA and molecules of methyl-esterified albumin in the MAK column. This latter class of complexes was suggested by experiments with columns that were built with kieselguhr alone. No radioactive RNA was retained on such columns, even after treatment with relatively high concentrations of PS.

**Kinetics of attachment of PS-treated RNA to nitrocellulose filters is shown in Chart 6B.** The binding pattern is similar to that of DNA (Chart 6A). The small quantitative differences in the degree of retention of the 2 nucleic acids may be due to the difference in molecular weights of the extracted DNA and cellular RNA's. Attachment of the PS-treated RNA to polypropylene (Nucleopore Corp.,

<table>
<thead>
<tr>
<th>ng DNA attached/million cells</th>
<th>Without DNase</th>
<th>With DNase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control (no carcinogen)</td>
<td>0.20</td>
<td>0.48</td>
</tr>
<tr>
<td>+ 0.04% PS</td>
<td>0.37</td>
<td>16.95</td>
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<tr>
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<td>0.26</td>
<td>0.72</td>
</tr>
</tbody>
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‡ DNA (or cells) mixed for 20 min with PS and incubated at 37°, followed by the addition of cells (or DNA) at time 0.

‡‡ Cells were exposed to the specified concentration of PS for 60 min at 37°, pelleted, washed once with TSM buffer, and resuspended in TSM; radioactive DNA was added, and the samples were incubated at 37°.

**Table 2**

**Effects of PS on binding of E. coli [³²P]DNA to Ehrlich ascites tumor cells (40 ng DNA per 10⁴ cells)**
DISCUSSION

Experiments summarized in this report indicate that PS interacts with cellular macromolecules producing complexes made of nucleic acids and nucleic acids and proteins. Such complexes are detectable by a variety of independent techniques. Thus, a fraction of PS-treated DNA sediments rapidly during centrifugation in sucrose gradients and is delayed during electrophoresis in polyacrylamide-agarose gels. Base composition and secondary structure of DNA seem to have little effect on the reaction between DNA and PS. Similarly, ionic strength and composition of the incubation medium and its pH have little effect on the depolymerization of DNA and subsequent formation of DNA megamolecules. A decrease in temperature during reaction, however, drastically reduces the rate of reaction; shearing of DNA before exposing it to PS similarly decreases the effects of this carcinogen.

DNA exposed to PS is retained to a higher degree than control samples on MAK columns. Since no such retention is seen in columns built with kieselguhr alone, we assume that the alkylated DNA reacts with the protein moiety of MAK columns. The conclusion that DNA-protein complexes may be formed under those conditions is further strengthened by experiments in which DNA was alkylated in the presence of albumin. Increased apparent molecular weight of such DNA is observed in alkaline sucrose gradients under conditions that do not induce any change in sedimentation pattern with albumin or PS alone. Moreover, the DNA-protein complexes may be detected by the change in density in either of the 2 compounds. Thus the density decrease of [32P]DNA centrifuged until equilibrium in CsCl after incubation with albumin and PS, indicates the formation of such artificial nucleoproteins. The magnitude of the density shift suggests that as much as 50% of the complex has been made up of albumin. The chemical bonds involved in the formation of the DNA-DNA and DNA-protein complexes seem to be relatively strong since neither high concentration of salts, nor high pH, nor ionic detergents are able to dissolve them.

Cellular RNA exposed to PS shows an increased rate of sedimentation through sucrose gradients and is retained on MAK columns. There is also a clear relationship between the molecular weight of an RNA species and its sensitivity to PS. While the elution pattern of tRNA is only slightly altered even at relatively high concentrations of PS, rRNA is significantly retained at low concentrations of this carcinogen. Moreover, the degree of retention of the larger rRNA is higher than that of the smaller RNA molecule.

In the presence of relatively low concentrations of PS, DNA binds to microsomal membrane more than the untreated control DNA. This increase in the amounts of DNA associated with the membrane band in CsCl density gradient experiments is related to the dose of the chemical and the length of exposure. Moreover, it correlates well with the degree of increase in the membrane density in CsCl. At higher concentrations of PS, however, both the membrane density and its ability to attach DNA are greatly decreased. This rather puzzling observation may perhaps be explained by our earlier studies on the effects of other biologically active chemicals on microsomal membrane (16). The membrane exposed to certain neuropharmacological agents loses part of its proteins during subsequent centrifugation in CsCl gradients. This loss may be detected by measuring the relative proportions of phospholipids and proteins in the treated membrane material isolated from CsCl gradients. After treatment with such diverse drugs as dopamine, tubocurarine, and tetracaine these proportions were significantly altered, in parallel with a decrease in membrane density and, in some cases, an alteration of its affinity toward DNA. It seems conceivable, therefore, that a similar mechanism may be involved during treatment of microsomal membrane with a high concentration of PS, leading to the release of certain membrane proteins acting as specific receptors for DNA. In fact, we have observed at high concentrations of PS a significant increase in the protein content in the gradient below the visible membrane band, with a concomitant decrease in proteins in the band (unpublished).

A related phenomenon was observed in a series of experiments designed to test the attachment of DNA to Ehrlich ascites tumor cells. The increased binding and, presumably, penetration of DNA in the presence of PS is not limited to this particular chemical. Experiments that will be published separately indicate that all ultimate carcinogens tested to date share this activity. We do not know at present whether the drastically increased ability of exogenous nucleic acids to enter the target cell may be in any way involved in the process of tumor induction by carcinogens. It seems likely that the increased binding of DNA to the plasma membrane of Ehrlich cells represents another aspect of the same phenomenon of enhanced reactivity of PS-treated nucleic acids, reflected in their increased affinity towards various cellular macromolecules and organelles.

We do not yet know the nature of the covalent-like bonds formed by PS that are responsible for the induction of macromolecular complexes such as the ones described in this report. As we have pointed out earlier (24), some secondary chemical reactions may be involved, such as depurination of nucleic acids, recently suggested by Verly (33). This question clearly requires further study.

While it is difficult to prove rigorously that any particular chemical alteration caused by a carcinogenic agent is the ultimate cause of the subsequent malignant transformation, we believe that the macromolecular complexes produced by some of the carcinogens deserve further attention. As we have suggested elsewhere (24, 27) it is unlikely that the known processes of cellular repair could effectively cope with the great variety of such complexes created by BPL or PS and remove them from a cellular chromosome. It seems likely, therefore, that these structures could interfere with normal DNA replication and chromosomal division and transcription, leading to a variety of phenotypic and genetic alterations in the injured cell.
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REFERENCES

Macromolecular Complexes Produced by 1,3-Propanesultone