Studies on Macromomycin, An Antitumor Protein*

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Macromomycin is a protein isolated from the culture filtrate of Streptomycyes macromomyceticus. It is an antibiotic and also cytotoxic to a broad spectrum of carcinoma cells, the ID₅₀ for P388 leukemia cells being $1 \times 10^{-9}$ m. Macromomycin binds rapidly and tightly to the P388 cell membrane and the eventual death of the cell cannot be reversed by either washing the toxin away or treating the cell with trypsin. The cytotoxicity does not appear to be specific for any phase of the P388 cell cycle.

Macromomycin is a single polypeptide, pI 5.38, devoid of methionine and arginine residues and contains 4 cysteine residues joined by two intramolecular disulfide bonds. The cytotoxicity results in inhibition of DNA, RNA, and protein synthesis in P388, the latter inhibition occurring a few hours after the inhibition of nucleic acid synthesis. The antibiotic and antitumor activities are destroyed rapidly by ultraviolet light, which gives a product that differs little in amino acid composition, molecular weight, and antigenic property, but can be separated from the native macromomycin by ion exchange chromatography. It is proposed that macromomycin has an ultraviolet-sensitive prosthetic group upon which much of the biological activity is based.

Macromomycin, a protein-antibiotic produced by Streptomycyes macromomyceticus (1), has been shown to be an effective anti-tumor agent in vivo against P388, L1210, B16, and Lewis lung carcinoma cells (2, 3). In tissue culture this protein is cytotoxic to a variety of transformed cell lines such as Ehrlich tumor cells, sarcoma 180, HeLa, and B16 cells (4, 5). It was reported earlier that macromomycin partially inhibits the incorporation of [³H]thymidine but not [³H]uridine or amino acids into biopolymers in sarcoma 180 cells (4). Furthermore, this partial inhibition of DNA synthesis as well as its cytotoxicity could be abolished by treatment of the cells with trypsin soon after their exposure to macromomycin (4). Similar observations were described in studies with B16 cells (5). Nevertheless, the primary cause(s) for cytotoxicity of macromomycin is still not clearly understood. A delineation of the molecular processes for these activities of macromomycin would be expected to give important information regarding some role of the plasma membrane in malignant cell proliferation.

Most of these earlier studies were carried out using partially purified macromomycin. Recently Yamashita et al. (6) reported the preparation of pure macromomycin using salt precipitation, DEAE-Sephadex column chromatography, and gel filtration. In the course of similar studies reported here it was noted that the pure macromomycin so isolated had somewhat different composition and properties from those reported by Yamashita et al. (6). This report is concerned further with photoactivation of macromomycin and the effect of macromomycin on the synthesis of macromolecules in synchronized and unsynchronized cultures of P388.

**MATERIALS AND METHODS**

Materials—Dried culture filtrates of S. macromomyceticus (purity of macromomycin about 1%) were obtained from Dr. John D. Douros, Natural Products Section, DR & RP, NCI. DE52, a DEAE-cellulose anion exchanger, was purchased from Whatman, Bio-Gel P-60 from Bio-Rad, ampholine pH 4 to 6 from LKB, [³H]amino-acids, [³H]uridine, and [³H]thymidine from Amersham-Searle, Soluene 350 and Dimilume 30 from Packard Instrument Co. All proteins used for molecular weight determination were the purest available from commercial sources. All other materials were of reagent grade quality and obtained from standard sources.

Analytical Methods—Disc gel electrophoresis was carried out using 7.5% acrylamide gel prepared in Tris, pH 8.8, and using an electrode buffer of Tris-glycine (pH 8.3) as described by Davis (7). Sodium dodecyl sulfate-acrylamide gel electrophoreses were carried out as described by Weber and Osborn (8) on 10% gels and by Laemmli (9) on 12.5% gels. Proteins were stained with Coomassie brilliant blue. Glycoproteins were detected by staining with periodate-Schiff reagent as described by Zacharius et al. (10). Electrophoresis experiments were carried out using an LKB 8101 electrophoresing column. A 2% standard LKB mixture was used for the pH 4 to 6 range. Focusing was carried out at 0°C and under 300 V for at least 48 h. Ultraviolet absorbance spectra were obtained with a Cary 15 spectrophotometer; other spectrophotometric measurements were done with a Beckman model DU spectrophotometer.

Protein was determined by the Lowry method (11) using a macromomycin standard. For amino acid analyses, the proteins were hydrolyzed with either 6 N HCl or 3 N p-toluene sulfonic acid containing 0.2% 3-(2-aminoethyl)indole (12) for 24, 48, and 72 h at 110°C. Cystine and/or cysteine were determined as cysteic acid after oxidation of samples with performic acid (13). The protein hydrolysates were analyzed using a model 120C Beckman amino acid analyzer.

Sulfhydryl groups were determined with 2,2'-dithiodipipyridine as described by Grassetti and Murray (14). Disulfide bonds were determined by analyzing for sulfhydryl groups before and after reduction of the protein samples with mercaptoethanol in 8 M urea. The reduced protein was precipitated by adding 25 volumes of a cold mixture of acetone and 1 N HCl (29:1) (15) and the precipitate was washed three times with this solvent mixture.

**Tissue Culture**—KB (derived from human epidermoid carcinoma), HTC (derived from rat hepatoma), and HeLa cells were maintained and subcultured as described previously (16). Leukemia P388 cells were kindly provided by Dr. Broome of Arthur D. Little, Inc., and

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**References**

were maintained as a suspension culture in Fisher's medium supplemented with 10% horse serum. To test cytotoxicity, cells dis-associated from stock monolayer culture by EDTA treatment, or cells from suspension cultures, were diluted in fresh growth medium to a density of $1 \times 10^6$ cells/ml. Typically, 3.5 ml of these diluted cultures were mixed with a 0.1-ml aliquot of 8 mm phosphate-buffered saline (130 mm), pH 7.4, containing various concentrations of the toxin in 12-ml culture tubes. After 2 h of incubation at an inclined angle of 10°, cellular protein was determined by the microbiuret method of Gooch (17). Tubes receiving no toxin or cells served as cell-free controls.

A thymidine block in a concentration of 2 mm for 20 h was used to synchronize leukemia P88 cells at the $G_0/S$ boundary (18). DNA synthesis was estimated by measuring $^3H$thymidine incorporation into that fraction of the cell that was insoluble in 5% trichloroacetic acid. Cell cultures (5 ml) at a cell density of about $5 \times 10^7$ cells/ml were centrifuged at 2000 $g$ for 4 min. The cell pellet was suspended in 1 ml of growth medium containing about 2 $\mu$Ci of $^3H$thymidine. After 15 min of incubation, 5 ml of cold phosphate-buffered saline containing 1 mm unlabeled thymidine were added. The cells were pelleted and washed twice with 5% trichloroacetic acid solution. $^3H$activity was measured in samples digested with Soluene 350. Diam- lume 30 was used as the scintillation fluid. $^3H$Uridine and $^3H$-amino-acids mixture were used to measure RNA and protein synthesis, respectively, in unsynchronized cell cultures. The same procedures used for DNA were employed except that for protein synthesis, cells were suspended in 1 ml of 8 mm phosphate-buffered saline (130 mm), pH 7.4, containing 2 $\mu$Ci of a mixture of $^3H$-amino-acids.

Macromomycin Antibody—Macromomycin (5 mg) in 1 ml of H$_2$O was mixed with 1 ml of Freund's complete adjuvant using a Multi- churn. Initial footpad immunization was followed 30 days later by an intracutaneous immunization with the same antigen mixed with Freund's incomplete adjuvant. Blood samples were drawn weekly for 3 weeks following the booster immunization. Immunodiffusion tests were carried out on 2% agar gels prepared in 8 mm phosphate-buffered saline (130 mm), pH 7.4.

Antimicrobial Activity—The concentration of macromomycin in a sample was estimated by the potency of antimicrobial activity against Staphylococcus aureus, measured by diffusion assays in Mueller-Hinton agar plates. The sample, usually in a 5-$\mu$l aliquot, was applied to a 3-mm paper disc placed on the agar plate, which had been seeded evenly with the microorganism. After incubation overnight at 37°C, a plot of the diameters of clear zones of no growth and the log of macromomycin concentrations shows a linearity up to about 2 $\mu$g of the pure macromomycin.

Purification of Macromomycin—Macromomycin was dissolved in a deaerated buffer and irradiated in a closed silicon cell. Illumination was carried out by a short wave ultraviolet lamp ("Mineralite") providing 240 microwatts/cm$^2$ of 254 nm radiation at a distance of 15 cm.

RESULTS

Purification of Macromomycin—All operations were carried out in the dark. General purification schemes were based on our preliminary observations that macromomycin binds to a cellulose anion exchanger equilibrated at pH 8.0 with Tris buffer but not to the same exchanger equilibrated with sodium phosphate buffer. Typically, a 2-g sample of the dried culture filtrate of S. macroomyceticus was dissolved in 40 ml of 0.01 M sodium phosphate buffer, pH 8.0. The solution was added to a column (15 x 5 cm) of DE52 equilibrated with 0.01 M sodium phosphate buffer, pH 8.0. The column was eluted with the equilibrating buffer until all the macromomycin had been displaced, requiring approximately 600 ml of effluent. The effluent containing macromomycin was dialyzed against water overnight and the nondialyzable solution was freeze-dried. The resulting crude macromomycin was dissolved in 20 ml of 0.01 M Tris hydrochloride buffer, pH 8.0, and added to a column (45 x 2.2 cm) of DE52 that had been equilibrated with the Tris-dissolving buffer. The column was eluted with this same Tris buffer until the proteins not bound to the resin were removed, which required approximately 500 ml of the eluting buffer. This was immediately followed by elution with a linear salt gradient formed from 500 ml of 0.01 M buffer, pH 8.0, and 500 ml of this same buffer that was 0.05 M in sodium chloride. Fig. 1 shows the elution pattern. Two principal peaks, A and $B$, were eluted and collected in fractions of about 15 ml by this gradient. The one (A) eluting with a peak concentration at about 0.038 M was concentrated to the macromomycin, which was confirmed by checking the antimicrobial activity of the fractions in the eluates against S. aureus. The fractions containing macromomycin were combined, concentrated by freeze-drying to about 5 ml, and chromatographed on a column (60 x 3.5 cm) of P-60 gel, eluting with 8 mm phosphate-buffered saline (130 mm), pH 7.4. The fractions eluting in the first peak were macromomycin, again being confirmed by the antimicrobial activity. The macromomycin-containing fractions were combined, dialyzed against water until free from salt, and freeze-dried; yield 20 to 30 mg.

Purity—Fig. 2 shows analytical disc gel electrophoresis of

![Fig. 1. Elution pattern from chromatography of the partially purified macromomycin (fractions shown in b and c of Fig. 2) on a column (45 x 2.2 cm) of DE52 equilibrated with 0.01 M Tris buffer, pH 8.0. The same buffer was used first to wash out unabsorbed materials. Then, the column was eluted with a linear gradient of sodium chloride from 0 to 0.05 M in 500-ml volume of buffer.](http://www.jbc.org/)

![Fig. 2. Acrylamide gel electrophoresis of various fractions obtained from column chromatographies of the culture filtrates containing macromomycin: a, crude macromomycin (4 mg was applied); b, fractions unabsorbed to DE52 column equilibrated with 10 mm sodium phosphate, pH 8.0 (1 mg each was applied); d and e, fractions unabsorbed to DE52 column equilibrated with 10 mm Tris, pH 8.0; f, macromomycin fraction (Fig. 1A) (50 $\mu$g); g, the second major anionic fraction (Fig. 1B) (50 $\mu$g); h, a mixture of A and B peaks in equal amounts; i, macromomycin fraction (Fig. 1A) (25 $\mu$g) on sodium dodecyl sulfate-acrylamide gel as described by Weber and Osborn (8). See "Materials and Methods" for details.)
representative fractions obtained during purification. It is seen that the macromomycin fraction from the DE52 column, the first major anionic fraction (A), migrates as a single band on analytical gel and 10% sodium dodecyl sulfate gel (Fig. 2). Nevertheless, upon column chromatography of the fraction over P-60 gel an ultraviolet-absorbing impurity was separated in the lower molecular weight region without impairing the antimicrobial or cytotoxic activities of the major fraction. Ioselectrofocusing of the final fraction yields a single peak with pI 5.38.

Rabbit antibody preparations against the macromomycin showed a single band of precipitate in reaction with not only the antigen, but also the culture filtrates (starting material) and a sample of macromomycin purified earlier by Chimura et al. (1) and Yamashita et al. (6). The precipitin lines in the double diffusion tests of these antigens as described by Ouchterlony (19) showed a pattern of antigenic identity. The immunoequivalence points of the crude culture filtrate, the macromomycin of Umezawa, and the present macromomycin samples against the preparation of rabbit antibody were 2500 µg/ml, 126 µg/ml, and 02 µg/ml, respectively, which is consistent with the improved purity of the present preparation of macromomycin. Similar relative tests for purity were demonstrated by the determination of antimicrobial activities against S. aureus.

Molecular Weight and Characteristics-The mobility of macromomycin on 12.5% gels in the presence of sodium dodecyl sulfate (9) indicates that the molecular weight of the protein is approximately 13,000 to 15,000. The mobility of macromomycin is clearly greater than myoglobin (18,700) but almost indistinguishable from that of cytochrome c (12,700) or lysozyme (14,100). Deviations of as much as 20% from the expected value are known to occur for this lower molecular weight range even under carefully controlled gel electrophoresis (20). The mobility of the protein on a column (1.5 x 66 cm) of Sephadex G 100 indicated a molecular weight range similar to that obtained from the sodium dodecyl sulfate gel electrophoresis. Macromomycin consists of a single polypeptide.

Analyses of macromomycin hydrolysates with 3 N p-toluenesulfonic acid and extrapolation of the hydrolysis value to zero time indicate that one tryptophan residue is present. Table I shows the amino acid composition of macromomycin thus obtained. This amino acid composition supports a molecular weight of approximately 13,800.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Number of residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>4</td>
</tr>
<tr>
<td>Histidine</td>
<td>2</td>
</tr>
<tr>
<td>Tryptophan*</td>
<td>1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>9</td>
</tr>
<tr>
<td>Threonine</td>
<td>21</td>
</tr>
<tr>
<td>Serine</td>
<td>12</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9</td>
</tr>
<tr>
<td>Proline</td>
<td>6</td>
</tr>
<tr>
<td>Glycine</td>
<td>21</td>
</tr>
<tr>
<td>Alanine</td>
<td>21</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>4</td>
</tr>
<tr>
<td>Valine</td>
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</tr>
<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
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</tr>
<tr>
<td>Tyrosine</td>
<td>1.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3</td>
</tr>
</tbody>
</table>

* Hydrolysis with 3 N p-toluenesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

The only residue we have difficulty in rounding up.

![Figure 3](http://www.jbc.org/)

**Figure 3.** Ultraviolet absorbance spectra of macromomycin in H₂O, 0.1 N HCl, and 0.1 N NaOH.

The antimicrobial activity of macromomycin was almost completely protected from the photoinactivation by adding p-aminobenzoate or to a lesser extent by adding deoxycholate. The protection is probably due to radical scavenging.

The samples of ultraviolet-irradiated macromomycin were also tested for their activity to inhibit DNA synthesis of transformed cell lines, which is another known biological activity of macromomycin (4). DNA synthesis was measured by the incorporation of [³H]thymidine into the nuclei of cultures of P388 synchronized at the G_/S boundary. In such synchronized cultures of P388 a maximum incorporation of [³H]thymidine was observed 4 h after their release from a
thymidine block. Fig. 5 shows the time course profiles of [\(^{3}H\)]thymidine incorporation in the synchronized cultures exposed to 4 \(\mu\)g/ml of macromomycin, illuminated for 0, 5, 10, and 20 min, immediately after the release of the thymidine block. The culture containing the sample irradiated for 20 min shows 95% of the control value (without macromomycin) in its thymidine incorporation measured at the 4th h. Apparently, ultraviolet irradiation of macromomycin causes a simultaneous destruction of both the antimicrobial activity and the ability to inhibit DNA synthesis by a transformed cell line, P388.

Comparisons in molecular properties were made between macromomycin and the sample irradiated with ultraviolet light for 20 min. The latter retained less than 10% of the antimicrobial activity of the former. The samples, about 0.5 mg each, were added to a column (1.4 \(\times\) 24 cm) of P-40 gel separately or as a mixture. On elution with 8 mM phosphate-buffered saline (130 mM), pH 7.4, no significant formation of either higher or lower molecular weight species were detected.

Even when co-chromatographed, only a single peak was observed with a normal distribution of the antimicrobial activity. A similar conclusion was reached from studies on the mobilities of the samples on 12.5% sodium dodecyl sulfate gel (9). A difference was observed, however, on chromatography of the samples on a column (1 \(\times\) 18 cm) of DE32 equilibrated with 0.01 M Tris, pH 8.0, and elution by a linear gradient from 50 ml of the Tris buffer to 50 ml of the buffer that was 0.1 M in NaCl. The samples, when chromatographed as an equal mixture, were resolved into two slightly overlapping peaks, the front-running peak being devoid of the antimicrobial activity. Nevertheless, there is no difference in mobilities between macromomycin and the inactivated macromomycin on analytical disc gel electrophoreses run as described by Davis (7). The amino acid compositions were examined in the samples hydrolyzed with 6 N HCl or 3 N p-toluene sulfonic acid for 24 h. The photoactivated sample retained the same amounts of histidine, tyrosine, phenylalanine, and tryptophan as those of macromomycin. Disulfide bonds were examined by titration with 2,2'-dithiodipyridine after full reduction of the samples or by analyzing cysteic acid content after oxidation with performic acid (13). After irradiation of macromomycin for 20 min, 92% of the cystine and 95% of the disulfide bonds remained as determined by amino acid analysis or titration, respectively, but there was an 87% loss of the antimicrobial activity. After 30 min of irradiation virtually all the antimicrobial activity was lost with little further change in the cystine or disulfide content of the protein. Furthermore, immunodiffusion tests with the antiserum against macromomycin shows the same equivalence points for the macromomycin irradiated for 20 min, and a pattern of coalescence is seen between the macromomycin and its inactivated derivative on Ouchterlony double-diffusion plates. Hence, we cannot exclude the possible presence of a prosthetic group in macromomycin.

**Cytotoxicity**—The cytotoxic effect of macromomycin on several established culture cell lines was examined. The cytotoxicity, expressed as the ID\(_{50}\) in micrograms/ml, for KB, HTC, P388, and HeLa was 8 \(\times\) 10^{-7}, 8 \(\times\) 10^{-6}, 2 \(\times\) 10^{-6}, and 4 \(\times\) 10^{-6}, respectively. Among the sensitive cells, P388 was chosen for further studies. This cell line has been used as an initial testing system for screening antitumor drugs by NC1 (21). We have examined the effect of the macromomycin on DNA synthesis in synchronized cells at the G1/S boundary as well as in unsynchronized cultures. Fig. 6 shows the effect of varying macromomycin concentrations on [\(^{3}H\)]thymidine incorporation measured 4 h after the release from a thymidine block, the time of peak DNA synthesis. Macromomycin was added to the culture immediately after the release of the thymidine block. Macromomycin at a concentration of 0.3 \(\mu\)g/ml causes a 50% reduction of [\(^{3}H\)]thymidine incorporation. More than a 95% inhibition was observed at concentrations above 2 \(\mu\)g/ml. This contrasts to the previous report (4) that only the partial inhibition of DNA synthesis in sarcoma cells was observed even at a level of 100 \(\mu\)g/ml of macromomycin/ml of the culture. Fig. 7 shows the effect of incubation periods of the synchronized and unsynchronized cells with 8 \(\mu\)g/ml of macromomycin. Incubation of the cells for 30 min at the beginning of the S-phase in the synchronized cultures caused 95% reduction of [\(^{3}H\)]thymidine incorporation measured 4 h after the release of a thymidine block.

Unsynchronized P388 cultures in log phase (7 \(\times\) 10^{6} cells/ml) were incubated with 8 \(\mu\)g/ml of macromomycin. Within an hour 80 to 90% inhibition of DNA or RNA synthesis was
In a manner yet to be described, the binding of some cytotoxic agents to the plasma membranes of sensitive cells causes an inhibition of DNA synthesis. One such compound is the protein macromycin, the purification of which from the culture filtrate of S. macromomyceticus is complicated by the presence of similar, but biologically inactive, proteins in the crude isolates. One of these proteins is synthesized by the bacterium and another is the result of photoinactivation of macromycin (1). The purification of the complex mixture takes advantage of the fact that macromycin does not compete with phosphate at pH 8.0 on DEAE-cellulose, but is absorbed to this ion exchanger in the chloride form at low ionic strength. Fractionation of the absorbed material is accomplished by a salt gradient elution in which the photoinactivated macromycin is eluted with the leading edge of the macromycin, whereas the other protein impurity is eluted at a higher salt concentration than macromycin. Because of the sensitivity of macromycin to light, particularly in the ultraviolet, it is important to conduct all operations in the dark. The photoinactivated macromycin, unlike the other protein impurity, does not separate from native macromycin on gel electrophoresis and furthermore stains less intensely with Coomassie blue on the gel. It is not possible, therefore, to quantitate the proteins in this type of mixture by the Coomassie blue stain, although the proteins are clearly identified by scans at 280 nm of the unstained gels.

Macromycin is a single polypeptide of molecular weight around 13,800, which is in keeping with its behavior in sodium dodecyl sulfate gel electrophoresis and gel chromatography. Consistent with previous reports (1, 6) the polypeptide contains no methionine or arginine residues. There are two disulfide bridges and no free sulfhydryl groups in the native state. Macromycin is reduced by mercaptoethanol in 8.0 M urea, but it is not known if this form is biologically active. The weakly acidic property, pI 5.88, is predicted from the amino acid analysis, assuming that approximately one-half of the aspartyl and glutamyl residues are in the form of their amides. Tryptophan, determined by amino acid analysis following hydrolysis with 3 N p-toluene sulfonic acid, was a single residue in the polypeptide and tyrosine accounted for 1 or 2 residues, again as determined by amino acid analysis of acid hydrolysates. The mole ratio of these two residues by spectroscopic methodology is 1.6, following Goodwin and Morton (22), or 0.96 by the method of Benze and Schmid (23). Previous reports suggest 2 residues of tyrosine/mol of macromycin based in part on analyses of 6 N HCl acid hydrolysates at 3, 6, and 12 h, from which it was reported that all the tyrosine was released within 3 h and 50% was then destroyed after 12 h of hydrolysis (6). The present studies indicate that the short hydrolysis times released a peptide that chromatographed close to tyrosine, but which disappeared after 22 h of hydrolysis. The definitive amino acid composition of macromycin will result from more detailed structural studies.

Macromycin is cytotoxic to KB, P388, HTC, and HeLa cells in tissue culture. Preliminary studies also indicate that breast cancer cell lines SW 613 and MDA-MB-231 are equally sensitive to macromycin. Its action was studied in detail with P388, where it was noted that concentrations of 3 µg/ml or more of macromycin inhibited DNA synthesis to an extent of more than 95% in both unsynchronized cells and those synchronized at the G1/S interface by a thymidine block. From such a synchronized cell culture, the maximum incor-
poration of \textsuperscript{3}H\textit{ thyminidine, using a 15-min pulse, into DNA occurred after 3 to 4 h of release from the block.} The effect of macromomycin was to reduce the incorporation but not significantly the time course of DNA synthesis. The cell count in the control P388 culture began to increase after about 8 h and reached a maximum after 10 h. In contrast, the presence of macromomycin in concentrations of 0.1 \(\mu g/mL\) or more showed gradual decrease in the number of viable (as defined by vital dye exclusion) cells. By similar protocols using unsynchronized P388 cells, the incorporation of \textsuperscript{3}H\textit{uridine and \textsuperscript{3}H}amino-acid mixture was determined. Unlike studies with sarcoma 180 (4) and B16 (5), it was noted that the biosynthesis of RNA was inhibited, as was that of protein, but the latter more slowly (Fig. 7). No such inhibition of RNA or protein synthesis was noted previously (4, 5) and may be due to differences in the cell lines. It is known, for example, that P388 contains C-type viruses which may account for incorporation of \textsuperscript{3}H\textit{uridine, but this many be inhibited by macromomycin. However, were such the case, then macromomycin must pass through the plasma membrane and this is not supported by our studies to date.

Macromomycin loses its biological activity when exposed to light (1, 6). The present studies show that the rate of photoinactivation under the short wave ultraviolet of a "Mineralite" is \textit{first order} with a \(t_{1/2}\) of 6.4 min in 0.75 mM phosphate buffer. In artificial laboratory light the process was much slower, but after 24 h of such exposure a solution of macromomycin had no antibacterial activity. The inactivation is significantly delayed by the presence of 0.02% \(p\)-aminobenzoate or 0.02% deoxycholate (Fig. 4), supporting a free radical mechanism for the photoactivation process.

The product from the photoinactivation of macromomycin showed no change in molecular weight as determined by gel filtration or sodium dodecyl sulfate gel electrophoresis, only a delayed by the presence of 0.02% \(p\)-aminobenzoate or 0.02% deoxycholate (Fig. 4), supporting a free radical mechanism for the photoactivation process.

The present studies of the action of macromomycin on P388 cells are therefore in general agreement with the earlier studies on other cell lines (4, 5), the polypeptide binding very tightly and rapidly to the plasma membrane and producing an inhibition in DNA synthesis. Differences were noted, however, in that the extent of inhibition of DNA synthesis was more complete and was accompanied by the inhibition of RNA synthesis followed by a decrease in protein synthesis. Preliminary experiments also indicate that in contrast with other cells (4, 5), P388 cells can neither be completely rescued by treatment with 0.08% trypsin for 10 min after previous exposure of the cells to macromomycin for 30 min, nor can the binding of the macromomycin be prevented by previous treatment of the cells for 10 min with 0.08% trypsin. The P388 membrane receptors for macromomycin are either not accessible to trypsin or are not hydrolytically destroyed.

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