# Metabolic Effects of an Antibiotic, NSC-51954, on Susceptible and Resistant Tumor Cells[1]

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SUMMARY

An antibiotic of unknown structure, NSC-51954, produced a very significant prolongation of survival time in mice bearing the ascites cell forms of Ca-755, Ehrlich, and S-180, but had only slight effects on mice bearing L1210. A subline of S-180 resistant to this drug was selected to aid the study of the mechanism by which it acts. The resistance of the derived cell line was confirmed by survival tests and by measurements of total packed cell volume after the treatment.

The incorporation of exogenously administered guanine-8-$^{14}$C into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of the sensitive S-180 cells was strongly inhibited by a single i.p. injection of 50 μg/kg NSC-51954, which did not appreciably inhibit the incorporation of glycine-2-$^{14}$C into nucleic acids and protein. This inhibition of guanine-8-$^{14}$C utilization was maintained for more than 24 hr. Although the incorporation into nucleic acids of exogenously administered adenine-8-$^{14}$C, orotic acid-6-$^{14}$C and uracil-2-$^{14}$C was also inhibited in the sensitive cells, these inhibitions were moderate and recovery was earlier than with guanine. The same dose of this drug inhibited the incorporation of guanine-8-$^{14}$C into the nucleic acids of the resistant S-180 cells and of L1210 cells only moderately. The inhibitions found in less sensitive tumor lines were no longer evident after 12 hr, and the incorporation of the labeled precursors was greater than in controls by 24 hr after the treatment.

*In vitro* experiments revealed that the incorporation of guanine-8-$^{14}$C into nucleic acids and the conversion of guanine-8-$^{14}$C into acid soluble nucleotides of the sensitive cells were both inhibited, whereas these reactions in the resistant cells were accelerated. Guanosine-5′-phosphate was formed at the same rate by the guanylic pyrophosphorylase reaction in cell free extracts from the sensitive and resistant S-180 cells, and the reaction was not affected by treating with NSC-51954.

The observed inhibition in the utilization of guanine for nucleic acid synthesis was correlated with tumor response in sensitive and resistant tumor cell lines.

A number of antibiotics have been reported to have carcinostatic properties, and the mechanisms of their metabolic effects have been studied by many investigators (3, 4, 9–11). Among those exhibiting interesting activity was an antibiotic of unknown structure, NSC-51954, found by workers in the Abbott Laboratories (13). Some information has been presented about its biologic and histologic effects (8, 13), but no information was available about its metabolic effects. The purpose of this paper is to present information on the mechanism of action of NSC-51954.

A number of anti-tumor agents, some of natural occurrence and some obtained by synthesis, are known to inhibit the metabolic pathways involved in nucleic acid synthesis. For this reason the metabolic effects of this drug on nucleic acid and protein synthesis were investigated first. Furthermore, drugs often have a multiplicity of metabolic effects of varied significance. It is therefore helpful in a study of mechanism to compare the metabolic differences in drug response of susceptible and resistant tumor cells.

Several tumor lines were tested to assess sensitivity to this drug. Among them, an S-180 ascites cell line was very sensitive and L1210 had low sensitivity. Besides these tumors, a subline of S-180 resistant to this drug was induced by the repeated passage of S-180 cells in mice treated with NSC-51954. After 10 such transfers, a suitable level of resistance became evident.

## MATERIALS AND METHODS

*Animals and tumors.*—Both 6-thioguanine (TG)-sensitive and -resistant sublines of Ehrlich, L1210, and S-180

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were transplanted as described previously (7). Adenocarcinoma 755 (Ca-755) was grown in a female BAF1 mouse (C57Bl/6J ♀ × $A^5$ ♂ F1).

A subline of S-180 resistant to NSC-51954 was induced by repeated passage in treated mice; the mice were treated by i.p. injection with 50 μg/kg of NSC-51954 once a day for 3 successive days, starting 24 hr after the inoculation with tumor cells, and the treated cells were transplanted every 7 days. This procedure was continued for 10 transfers. To maintain the resistance, this line was routinely treated once with 50 μg/kg of NSC-51954 1 day after the transplantation. Resistance of this line was confirmed by survival and total packed cell volume tests at frequent intervals throughout the transplantation history.

*Survival, total packed cell volume, and solid tumor tests.*—For the survival tests, 0.05–0.2 mg/kg of NSC-51954 dissolved in physiologic saline solution was administered i.p. either once or twice a day, starting 24 hr after the transplantation. Fifty-day survivors were calculated as if they had survived only 50 days.

For the total packed cell volume tests, ascites tumor cells treated as above, were collected by washing the peritoneal cavity 3 times with normal saline 1 day after the final treatment, and measuring the packed cell volume after centrifugation at 1400 × *g* for 2 min.

Each tumor was inoculated s.c. ($10 \times 10^6$ cells). Four days later i.p. treatment was begun and repeated for 3 days. Then the 7-day-old tumors were extirpated and weighed on a torsion balance.

*Schedule of drug administration.*—Ascites tumor cells (5–6 days old) were used for the biochemical studies. Each mouse received NSC-51954 i.p. at the indicated dose (0.05–0.5 mg/kg); control mice received the same volume of saline.

At the prescribed time 0.5 μmole guanine-8-$^{14}$C (0.5 μc), 1.0 μmole glycine-2-$^{14}$C (1.0 μc), 0.5 μmole orotic acid-6-$^{14}$C (1.5 μc), 0.5 μmole uracil-2-$^{14}$C (1.0 μc), and 0.5 μmole adenine-8-$^{14}$C (0.8 μc) were injected i.p. in 0.2–0.5 ml of normal saline. Twenty minutes were allowed for metabolic utilization of guanine, and 60 min for the other substrates.

*Extraction of acid-soluble nucleotides.*—The ascites cells were withdrawn by Pasteur pipets and centrifuged, and the fluid was discarded. The tumor cells were extracted with 5 volumes of cold 0.2 M perchloric acid. The acid insoluble residue was centrifuged and washed once with 2 volumes of cold 0.2 M perchloric acid. The combined supernatants were neutralized with KOH, chilled, centrifuged to remove potassium perchlorate, and passed through a Dowex 1-formate column, 10 by 30 mm. Each column was eluted with 10 ml of 6 M HCl. Each such eluate was evaporated *in vacuo*, and this dried sample was used for the further study of the acid-soluble nucleotides.

*Extraction and estimation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).*—RNA and DNA were extracted by the following modification of the method of Tyner *et al.* (12): Phospholipid was removed from the acid-insoluble fraction by extraction with warm alcohol-ether. The precipitate was suspended in cold 10% NaCl, neutralized with small quantities of 0.1 N NaOH, and centrifuged. The precipitates were resuspended in 5 volumes of 10% NaCl and placed in a boiling water bath for 45 min; neutrality was maintained with saturated $NaHCO_3$. The suspensions were centrifuged, and the precipitates were extracted with 3 volumes of boiling 10% NaCl for 30 min. To the combined extracts, 3 volumes of cold ethanol were added. The precipitated nucleic acids were collected by centrifugation, dissolved in 2.5 ml of 0.1 N NaOH, and incubated at 37°C for 18 hr. After chilling, 1 N HCl was added to the solution to make it 0.1 N with respect to HCl. Then the hydrolyzed RNA was separated from the DNA precipitate by centrifugation in the cold. The precipitated DNA was rapidly washed with 5 volumes of cold 0.1 N HCl, suspended in 1 N HCl, and heated in a boiling water bath for 30 min. Aliquots of these RNA and DNA solutions were plated and counted by the standard procedures in a Nuclear-Chicago gas flow end-window counter (30% efficiency) and corrected for self-adsorption. RNA was estimated by the orcinol reaction method (5) and DNA by a modified diphenylamine reaction (1).

*Protein fraction.*—The protein fraction was prepared from the residue after the extraction of nucleic acids by a method based on that described earlier (6). The residue was washed twice with acetone and dried *in vacuo*. The dried samples were ground to fine powders, suspended in 25% ethanol and plated on aluminum discs. These plates were dried, weighed, and counted in a gas flow counter. Their specific radioactivities were calculated after correction for self-absorption.

Protein concentrations in the cell-free extracts used for enzyme assays were estimated after the extraction of the acid-soluble components and nucleic acids, using a 1.0 ml aliquot of the solution.

*Isolation of purine components.*—The acid-soluble nucleotides were dissolved in 0.4 M perchloric acid and hydrolyzed by heating for 45 min. The acid-insoluble residue was also extracted and hydrolyzed with boiling 0.4 M perchloric acid for 30 min.

The extracted and hydrolyzed purine components from both acid-soluble fractions and nucleic acids were neutralized with KOH, centrifuged in the cold to remove potassium perchlorate and run through Dowex 50 columns, 5 by 30 mm. The columns were each eluted with 10 ml of 6 N HCl. The eluates were all evaporated *in vacuo* in desiccators over sodium hydroxide. The dried residue from each was taken up in 0.1 ml of 0.1 N HCl and put on Whatman No. 1 filter paper. Descending chromatography was carried out with 5% $Na_2HPO_4$. The spots on the paper were located with an ultraviolet lamp, and each was cut out and eluted with 3.3 ml of 0.1 N HCl. The concentrations of adenine and guanine in these eluates were measured in a model DU Beckman spectrophotometer, and the specific radioactivity of each was calculated.

*Incorporation of guanine-8-$^{14}$C into ascites cells* in vitro.—Ascites tumor cells, 5–6 days old, were withdrawn and centrifuged. The fluid was discarded and the cells were washed twice with saline. These washed cells (approximately 60 mg dry weight per flask) were incubated at 37°C for 20 min in an atmosphere of 5% $CO_2$ and 95% $O_2$ in 50-ml Erlenmeyer flasks, each containing 10 ml of

Robinson's bicarbonate glucose solution (6), 1.0 μmole guanine-8-$^{14}$C (1.0 μc), and 2.5 μg NSC-51954 in a final volume of 12 ml. The reaction was terminated by chilling and adding 1.0 ml of 2.6 M perchloric acid. The acid-soluble nucleotide and nucleic acid fractions were prepared as described above.

*Enzymatic synthesis of guanosine-5'-phosphate.*—To assay for the enzymatic synthesis of guanosine-5'-phosphate, the method of Ellis and LePage (2) was used with guanine-8-$^{14}$C as substrate. The cells were disrupted with a Potter-Elvehjem homogenizer and centrifuged at 20,000 × *g* for 60 min. The supernatant was used as the enzyme source. A 0.10 ml portion of this solution (approximately 300 μg protein) was incubated for 1 hr at 37°C in a medium containing 50 μmoles Tris buffer (pH 7.6), 0.6 μmoles guanine-8-$^{14}$C (0.6 μc), 1.5 μmoles 5-phosphoribosyl-1-pyrophosphate, with or without 1.25 μg of NSC-51954, in a final volume of 1.0 ml. The reaction was terminated by chilling and adding 0.1 ml of 2.6 M perchloric acid. Aliquots of the reaction were spotted on the paper and descending chromatography was carried out with 5% $Na_2HPO_4$. The spots on the paper were located with an ultraviolet lamp and eluted with 0.1 N HCl for the counting of radioactivity.

*Investigations on peripheral leukocyte counts, bone marrow, and spleen.*—The number of circulating leukocytes was counted, by standard methods, with blood obtained from the tails of female Swiss mice, with and without pretreatment with NSC-51954. Each value represents the average of determinations on 5 mice.

Thirty minutes after the i.p. injection of guanine-8-$^{14}$C (1 μmole), bone marrow was collected from both femurs and tibias of each female Swiss mouse by flushing with cold saline into graduated centrifuge tubes. The spleen was extirpated from the same mouse in each case; each spleen was weighed and homogenized.

The acid-soluble fraction of bone marrow was extracted with 0.2 M perchloric acid and purine components of nucleic acids were extracted and hydrolyzed with boiling 0.4 M perchloric acid. These fractions from bone marrow were neutralized, plated, and counted in a gas flow counter. The acid-soluble nucleotides and nucleic acid purines were extracted from the spleens, as described above. Each value represents the average from 3 separate experiments.

## RESULTS

*Inhibition of tumor growth.*—At doses of 0.5 mg/kg or more, once a day, NSC-51954 produced severe toxicity and death in female Swiss mice. Doses of 0.05–0.1 mg/kg resulted in marked prolongation of the survival time of the mice bearing S-180 ascites tumors, without any body weight loss (Table 1).

At 0.1 mg/kg, twice a day for 6 days, starting 24 hr after the tumor transplantation, NSC-51954 was effective on the mice bearing both 6-TG-sensitive and -resistant sublines of Ca-755, Ehrlich and S-180; it was less effective on L1210 (Table 2).

It is evident from Table 3 that survival of mice bearing the sensitive line of S-180 was increased by more than 250% over that of controls, and that tumor growth found in total packed cell volume tests was suppressed strongly by the treatment with NSC-51954 at 0.05 mg/kg. In contrast, the line of S-180 resistant to this drug was not affected. The solid form of S-180 was not affected by the drug.

Based on the above findings, it was decided to use this drug at 0.05 mg/kg in the further study *in vivo*.

*Incorporation of C$^{14}$-labeled precursors into RNA, DNA and protein* in vivo.—Data concerning the influence of NSC-51954 on the utilization of precursors for growth of ascites tumors are given in Table 4.

The incorporation of exogenously administered guanine-8-$^{14}$C into both RNA and DNA of the sensitive S-180 cells

TABLE 1

COMPARISON OF EFFECTS OF NSC-51954 ON HOST AND TUMOR

| Dose (mg/kg) | Toxicity to Swiss Mice[a] | | | Effects on Sarcoma-180 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of doses | No. of deaths | Body weight change at end of treatment (%) | Average survival[b] (days) | Guanine-8-$^{14}$C incorporation[c] (%) | | Glycine-2-$^{14}$C incorporation[c] (%) | | |
| | | | | | RNA[d] | DNA | RNA | DNA | Protein |
| 0 (saline) | 6 | 0/5 | + 2.0 | 14.5 ± 2.8[e] | 100 | 100 | 100 | 100 | 100 |
| 0.02 | 6 | 0/5 | + 2.0 | | 108 | 115 | | | |
| 0.05 | 6 | 0/5 | + 2.0 | 37.6 ± 15.2 | 36 | 54 | 78 | 89 | 90 |
| 0.1 | 6 | 0/5 | ± 0 | 33.9 ± 14.1 | | | | | |
| 0.2 | 6 | 0/5 | − 2.0 | 30.0 ± 13.2 | 29 | 22 | 90 | 83 | 94 |
| 0.5 | 2 | 5/5 | −18.0 | 5.4 ± 1.1 | 13 | 12 | 56 | 79 | 77 |
| 1.0 | 2 | 5/5 | −16.0 | 3.8 ± 0.8 | | | | | |

[a] Toxicity to nontumor bearing mice. These mice were observed for 30 days.

[b] NSC-51954 was injected i.p. once a day for 6 days, starting 24 hr after the transplantation. Fifty-day survivors were calculated as surviving only 50 days.

[c] Guanine-8-$^{14}$C (0.5 μc, 0.5 μmole) and glycine-2-$^{14}$C (1.0 μc, 1.0 μmole), respectively, were injected i.p. 6 hr after the single treatment, and their incorporations were expressed as percent of control specific activity (cpm/mg RNA, DNA or protein).

[d] RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

[e] Standard deviation.

TABLE 2

SURVIVAL TIMES OF MICE BEARING ASCITES TUMORS TREATED WITH NSC-51954

Groups of 10 mice were used.

| Treatment | Ca-755 | Ca-755 R[a] | L1210 | L1210 R[a] | Ehrlich | Ehrlich TGRI[a] | S-180 | S-180 R[a] |
|---|---|---|---|---|---|---|---|---|
| Control | 14.2 ± 2.5[b] | 8.4 ± 0.9 | 6.9 ± 0.4 | 7.8 ± 0.5 | 15.2 ± 3.3 | 15.7 ± 3.0 | 16.3 ± 4.1 | 15.3 ± 3.6 |
| 0.1 mg/kg[c] | 36.9 ± 15.7 | 44.7 ± 8.5 | 9.9 ± 0.4 | 9.7 ± 0.6 | 33.9 ± 6.1 | 42.9 ± 9.2 | 36.1 ± 13.9 | 31.0 ± 9.6 |

[a] Subline resistant to 6-thioguanine.

[b] Average in survival days and standard deviation. Fifty-day survivors were calculated as surviving only 50 days.

[c] Treated i.p. twice a day for 6 days, beginning 24 hr after tumor transplantation.

TABLE 3

EFFECTS OF NSC-51954 ON THE SURVIVAL TIME AND GROWTH RATE IN ASCITES AND SOLID TUMORS

For the survival study, 10 mice were used for each group, in each of 3 experiments.

| Tumor | Treatment | Survival Time (days) | Total Packed Cell Volume[a] (ml) | Solid Tumor[b] (mg) |
|---|---|---|---|---|
| S-180 | Control | 14.7 ± 2.8 | 1.50 ± 0.26 | 243 ± 61 |
| S-180 | 50 μg/kg | 38.5 ± 14.1 | 0.20 ± 0.12 | 212 ± 56 |
| S-180 R[c] | Control | 14.6 ± 5.7 | 1.01 ± 0.20 | 241 ± 65 |
| S-180 R[c] | 50 μg/kg | 13.0 ± 3.3 | 1.26 ± 0.39 | 265 ± 44 |

[a] Each mouse was treated once a day for 6 days, beginning 24 hr after the transplantation, and ascites fluid was collected by washing the peritoneal cavity 3 times with normal saline solution 24 hr after the final treatment. Packed cell volume was measured after centrifugation at 1400 × *g* for 2 min. Each value indicates the mean and standard deviation obtained from 15 mice.

[b] $10 \times 10^6$ tumor cells were transplanted s.c. in each mouse. Four days later the treatment began and was given twice daily on the following 3 days. The 7-day-old tumors were extirpated and weighed on a torsion balance. Ten mice were used for each group.

[c] Induced subline of S-180 resistant to NSC-51954.

was strongly inhibited (about 75%) by a single i.p. injection of 0.05 mg/kg NSC-51954, which did not inhibit the incorporation of glycine-2-$^{14}$C into nucleic acids and protein. The inhibition of the utilization of guanine-8-$^{14}$C was maintained for more than 24 hr.

At the higher dose, 0.5 mg/kg, the incorporation of guanine was inhibited almost completely and that of glycine was moderately inhibited at 6 hr after the treatment (Table 1).

Although incorporation of exogenously administered adenine-8-$^{14}$C, orotic acid-6-$^{14}$C, and uracil-2-$^{14}$C into nucleic acids of the sensitive cells was also inhibited, the inhibitions were moderate and control incorporation rates were reached earlier than with guanine-8-$^{14}$C. A dose of 0.05 mg/kg NSC-51954 slightly inhibited the incorporation of guanine-8-$^{14}$C into nucleic acids of the resistant S-180 cells, but for a more limited time. The same dose of the drug inhibited the incorporation of guanine-8-$^{14}$C into nucleic acids of L1210 cells only moderately. These inhibitions by NSC-51954, found in the resistant or less susceptible tumor cells, had begun to recover within 12 hr after the treatment, and the incorporations were accelerated at 24 hr after the treatment.

*Incorporation of $^{14}$C-labeled precursors into purine com*

TABLE 4

INHIBITION OF INCORPORATION OF $^{14}$C-LABELED PRECURSORS INTO NUCLEIC ACIDS AND PROTEINS OF ASCITES CELLS TREATED WITH NSC-51954

| Tumor | Precursor[a] | RNA[b] | | | | DNA | | | | Protein | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 hr[c] | 12 hr | 24 hr | 48 hr | 6 hr | 12 hr | 24 hr | 48 hr | 6 hr | 12 hr | 24 hr |
| S-180 | Orotic acid-6-$^{14}$C | 35 | 30 | 18 | | 41 | 34 | 19 | | | | |
| S-180 | Glycine-2-$^{14}$C | 22 | 21 | −16 | | 11 | 16 | 8 | | 10 | 18 | 15 |
| S-180 | Uracil-2-$^{14}$C | 33 | 24 | 21 | | 49 | 53 | 18 | | | | |
| S-180 | Adenine-8-$^{14}$C | 40 | 18 | −27 | | 40 | 59 | −13 | | | | |
| S-180 | Guanine-8-$^{14}$C | 64 | 66 | 75 | 36 | 46 | 71 | 78 | 47 | | | |
| S-180 R | Guanine-8-$^{14}$C | 23 | −13 | −14 | | 19 | −32 | −17 | | | | |
| L1210 | Guanine-8-$^{14}$C | 34 | 23 | −19 | | 47 | 37 | −54 | | | | |

[a] Orotic acid-6-$^{14}$C (1.5 μc, 0.5 μmole), glycine-2-$^{14}$C (1.0 μc, 1.0 μmole), uracil-2-$^{14}$C (1.0 μc, 0.5 μmole), adenine-8-$^{14}$C (0.8 μc, 0.5 μmole) and guanine-8-$^{14}$C (0.5 μc, 0.5 μmole) were injected i.p. A 20-minute period was allowed for metabolic utilization of guanine-8-$^{14}$C and 1 hr for other substrates. Each value represents the average percent inhibition of incorporation obtained in 3 separate experiments having a maximum variation of ±15%. Cells from 2 mice were pooled for each analysis.

[b] RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

[c] Tumor-bearing mice were each given a single i.p. dose of 50 μg/kg of NSC-51954 and, at the indicated time after the treatment, $^{14}$C-labeled precursors.

TABLE 5

EFFECTS OF NSC-51954 ON THE INCORPORATION OF $^{14}$C-LABELED PRECURSORS INTO ACID-SOLUBLE (AS) PURINES AND NUCLEIC ACIDS (NA)

The methods of separation of purine components are described under "Materials and Methods." The cells from 2 mice were pooled for each analysis, and each value represents the average of 2–4 separate experiments having a maximum variation of ±15%.

| Tumor | Precursor[a] | Treatment | AS-adenine cpm/μmole | NA-adenine cpm/μmole | NA-guanine cpm/μmole |
|---|---|---|---|---|---|
| S-180 | Glycine-2-$^{14}$C | Control | 5,300 | 200 | 470 |
| S-180 | Glycine-2-$^{14}$C | 50 μg/kg | 4,910 | 180 | 490 |
| S-180 | Adenine-8-$^{14}$C | Control | 59,000 | 3770 | 470 |
| S-180 | Adenine-8-$^{14}$C | 50 μg/kg | 44,000 | 2700 | 470 |
| S-180 | Guanine-8-$^{14}$C | Control | 3,880 | 170 | 1270 |
| S-180 | Guanine-8-$^{14}$C | 50 μg/kg | 2,180 | 110 | 620 |
| S-180 R | Guanine-8-$^{14}$C | Control | 4,020 | 200 | 1050 |
| S-180 R | Guanine-8-$^{14}$C | 50 μg/kg | 3,530 | 170 | 950 |

[a] $^{14}$C-labeled precursors were injected i.p. 12 hr after treatment with 50 μg/kg of NSC-51954; a 20-min period was allowed for the metabolic utilization of guanine-8-$^{14}$C and 60 min for other precursors.

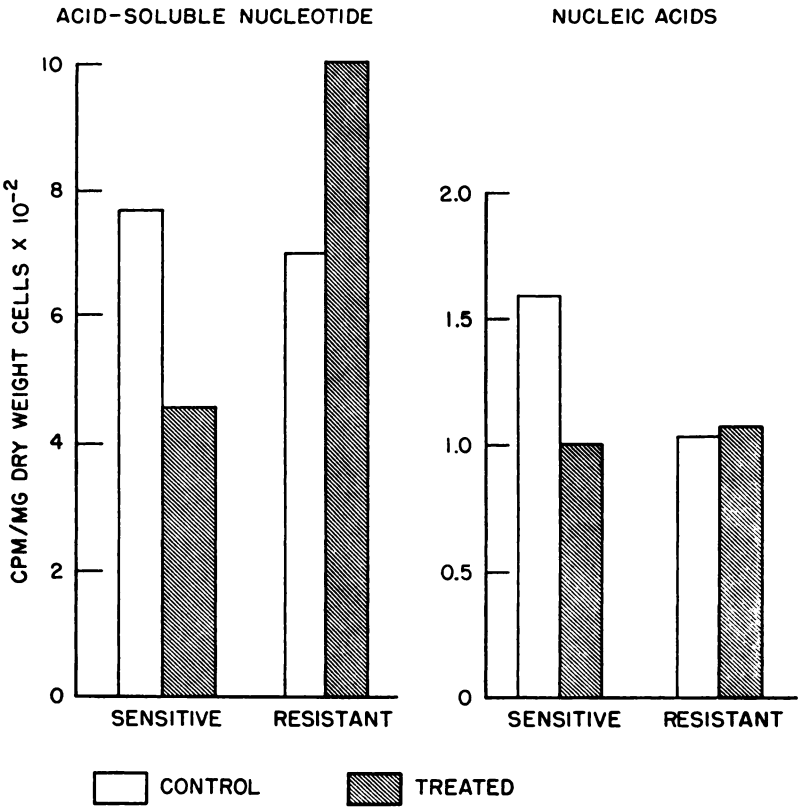


CHART 1.—Effects of NSC-5194 on the incorporation of guanine-8-$^{14}$C into the sensitive and resistant sublines of S-180 *in vitro*. Each aliquot of tumor cells (approximately 60 mg dry weight) was incubated for 20 min at 37°C in a medium containing 10 ml of Robinson's bicarbonate glucose medium (6), 1.0 μmole guanine-8-$^{14}$C, and 2.5 μg NSC-51954 in a final volume of 12 ml. The separations of acid-soluble and nucleic acid fractions were as described under "Materials and Methods."

Each bar represents the average of analyses of duplicate flasks in each of 4 separate experiments.

*ponents in acid-soluble nucleotides and nucleic acids.*— Twelve hours after the single i.p. injection of NSC-51954, the utilization of $^{14}$C-labeled precursors was investigated (Table 5). The conversions of guanine-8-$^{14}$C into nucleic acid guanine (NA-G) and into acid-soluble nucleotide adenine (AS-A) of the sensitive S-180 cells were inhibited by 51% and 44%, respectively (guanine components found in the acid-soluble nucleotides were so small in

amount and radioactivity that it was impossible to compare them under the conditions described here). On the other hand, those in the resistant cells were inhibited only about 10%.

The conversions of glycine-2-$^{14}$C into AS-A, NA-A, and NA-G of the sensitive cells were inhibited less than 10%.

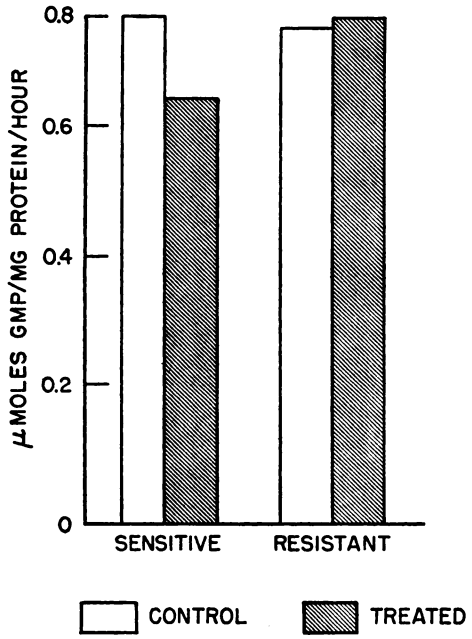


CHART 2.—Enzymatic synthesis of guanosine-5′-phosphate by cell-free preparations from S-180 cells sensitive and resistant to NSC-51954.

Aliquots (0.01 ml) of enzyme solution (approximately 300 μg protein) were incubated for 1 hr at 37°C in a medium containing 50 μmoles Tris buffer (pH 7.6), 0.6 μmoles guanine-8-C$^{14}$, and 1.5 μmoles 5-phosphoribosyl-1-pyrophosphate, with or without 1.25 μg of NSC-51954, in a final volume of 1.0 ml.

Each bar represents the average of analyses from 3 separate experiments.

*Incorporation of guanine-8-$^{14}$C into acid-soluble nucleotides and nucleic acids* in vitro.—*In vitro* study revealed that the incorporation of guanine-8-$^{14}$C into nucleic acids and the conversion into acid-soluble nucleotides of the sensitive cells was also inhibited moderately by the presence of 0.2 μg/ml NSC-51954 in the reaction medium (Chart 1).

Less guanine-8-$^{14}$C was incorporated into the nucleic acids of the resistant cells, and this incorporation was not inhibited by the same dose of the drug. The conversion into the nucleotides of the resistant cells was not decreased, but rather was accelerated.

*Enzymatic synthesis of guanosine-5′-phosphate.*—Not only the incorporation of guanine-8-$^{14}$C into nucleic acids but also its conversion to the acid-soluble nucleotides was inhibited by treatment with NSC-51954 *in vivo* and *in vitro*. Therefore, guanosine-5′-phosphate formation in the cell-free extracts from the sensitive and resistant cells was compared in the presence or absence of NSC-51954 (Chart 2).

Guanosine-5′-phosphate was formed at the same rate in the cell-free extracts from both the sensitive and resistant S-180 cells, and it was not affected by treatment with 1.25 μg/ml NSC-51954.

*Effects on leukocyte count, bone marrow, and spleen.*—The single i.p. injection of 0.05 mg/kg of the drug caused slight inhibitions of guanine-8-$^{14}$C incorporation into both acid-soluble and nucleic acid fractions of the spleen, and moderate inhibitions in the bone marrow, but these inhibitions were overcome 2 or 3 days after the treatment. Injection once a day for 6 days, at the same dose as above, did not result in leukopenia 24 hr after the final injection (Table 6).

## DISCUSSION

NSC-51954 strongly inhibited the incorporation of exogenously administered guanine-8-$^{14}$C into nucleic acids at a concentration which did not inhibit the incor-

TABLE 6

EFFECTS OF NSC-51954 ON LEUKOCYTE COUNT, BONE MARROW, AND SPLEEN

| Time after treatment[a] | WBC/cu mm[b] | Uptake of guanine-8-$^{14}$C into bone marrow[c] | | Incorporation of guanine-8-$^{14}$C into spleen[c] | |
|---|---|---|---|---|---|
| | | Acid-soluble (cpm/mg fresh wt.) | Nucleic acid (cpm/mg fresh wt.) | Acid-soluble (cpm/mg fresh wt.) | Nucleic acid (cpm/mg fresh wt.) |
| Control | 9,210 | 1.44 | 0.74 | 3.23 | 0.89 |
| 24 hr | 9,300 | 1.27 | 0.60 | 2.78 | 0.66 |
| 48 hr | 8,600 | 1.00 | 0.50 | 5.59 | 1.06 |
| 72 hr | 9,320 | 1.30 | 0.68 | — | — |
| 1 wk | 10,790 | 1.33 | 0.70 | 3.47 | 0.83 |
| 24 hr[d] (6 injections) | 8,740 | 0.67 | 0.48 | 3.37 | 0.53 |

[a] Each female Swiss mouse was given a single i.p. dose of 50 μg/kg of NSC-51954 and was sacrificed at the described time after the treatment.

[b] Leukocytes were counted according to standard methods after the aspiration of circulating blood from the tail. Each value represents the average of counts on blood from 5 mice.

[c] One μmole guanine-8-$^{14}$C was injected i.p. 30 min prior to sacrifice. The bone marrow and spleen were extirpated from 2 mice and pooled. Each figure represents the average value from 3 separate experiments.

[d] Treated i.p. once a day for 6 days.

poration of glycine-2-$^{14}$C into nucleic acids and protein. The inhibitions observed in the induced resistant S-180 cells and the less susceptible L1210 cells to NSC-51954 were slight or moderate, and normal rates were recovered earlier than in the sensitive cells.

These findings indicate that the utilization of preformed guanine for nucleic acid synthesis is the area of metabolism specifically damaged by treatment with NSC-51954. The carcinostatic responses correlate with this observation.

These results permit a focus on the area of metabolism in which NSC-51954 acts, but do not permit a conclusion concerning the specific step in the synthesis affected. Since tumor cells apparently can either use preformed purines or synthesize them *de novo*, it seems logical to combine treatment of this agent with others, such as azaserine, which interrupt purine synthesis *de novo*. Further studies are in progress on this possibility.

## REFERENCES


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