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ALTERATIONS INDUCED BY 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA (BCNU) IN DEOXYRIBONUCLEIC ACID SYNTHESIS IN NORMAL AND TUMOROUS TISSUES

IMPLICATIONS FOR OPTIMAL CHEMOTHERAPY

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Abstract—Alterations in DNA synthesis are induced by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in host target tissues and L1210 tumor. The timing of suppression and recovery of DNA synthesis was similar at the four doses studied (4, 8, 16 and 32 mg/kg); however, a dose–response effect was seen as the magnitude of suppression of DNA synthesis increased with increasing dosages. Of greater potential therapeutic importance, however, was that a pattern of suppression and recovery of DNA synthesis occurred in the host target tissues (bone marrow and gastrointestinal mucosa) which was different from that seen in the tumorous tissue, These differences in suppression and recovery of the normal and tumorous tissues were exploited for the prediction of dose schedules which resulted in improved survival of the tumor-bearing mice. The alterations in DNA synthesis induced by BCNU suggested an early fall in the *de novo* thymidine pool size. The delay in onset of DNA suppression suggests that the intact molecule is not the active principle, nor is there direct enzymatic blockade of DNA synthesis.

THE SCHEDULING of cancer chemotherapy has remained largely empirical as a result of a lack of knowledge of the mechanism of action of many of the antitumor agents employed and, possibly more important, a lack of appreciation of possible differences in the kinetic changes effected simultaneously in both the sensitive host target tissues and the tumor.

Previously, a study was performed in which changes in DNA synthesis in normal and tumorous tissues *in vivo* were followed simultaneously after the administration of the cycle-phase specific agent, cytosine arabinoside (Ara-C).[1] It was possible to demonstrate by this technique that patterns of suppression and recovery of DNA synthesis in the normal and tumorous tissues produced by cytosine arabinoside were sufficiently different to allow prediction of a dose schedule which would increase survival. 1,3-bis(2-Chloroethyl)-1-nitrosourea (BCNU) is a member of a relatively new class of chemotherapeutic agents, the nitrosoureas. These agents have been shown to be active against a wide spectrum of animal tumors,[2–4] and in subsequent clinical trials have demonstrated antitumor effect against Hodgkin's disease and non-Hodgkin's lymphoma[5,6] as well as a wide variety of solid tumors in man.[7–9]

BCNU has been shown by Wheeler and Bowden[10,11] to depress DNA synthesis and, although thought by many investigators to function by the mechanism of alkylation[12] or carbamylation,[13] its precise mechanism of action is unknown.[14] However, Wheeler and Bowden[10] have demonstrated that BCNU and a decomposition

product, 2-chloroethyl isocyanate, have been equally active as inhibitors of DNA nucleotidyltransferase *in vitro*, although inhibition of this enzyme could not be shown *in vivo*. In addition, they also demonstrated that BCNU had little effect upon the priming activity of DNA either *in vivo* or *in vitro*.[10] Therefore, it appeared possible that the inhibitory effect of BCNU on DNA synthesis might be exploited in a manner similar to that seen in studies with Ara-C[11] and methotrexate,[15] even though the agent was not cycle-phase specific, as were the previously studied agents.

As no convincing animal model presently exists for the unique delayed hematopoietic toxicity seen in man[16] following BCNU therapy, it was also our aim in this study to investigate the possibility that a late depression of DNA synthesis in the bone marrow might occur after BCNU administration, even though the animal might not manifest this effect with pancytopenia.

MATERIALS AND METHODS

BDF_1 female mice of an average weight of 20 g were obtained from Hazleton Laboratories (Falls Church, Va.) and used throughout these experiments. They were maintained in a constant temperature environment, in plastic cages, and fed laboratory Chow and water *ad lib*. L1210 murine leukemia carried intraperitoneally (i.p.) was used as the tumor source and transplanted to recipient mice by the injection of 1×10^5 L1210 cells i.p. BDF_1 female mice of comparable age and weight were utilized as normal controls. Experiments were carried out during the same time period each day and all mice were sacrificed by cervical dislocation.

Specific activity of the DNA. Studies requiring the use of tumorous mice were initiated at day 6 of tumor growth. All treatments were given i.p. at time zero. Six mice were left untreated and served as the zero-hr control group. At time zero, 1, 6, 12, 24, 36, 48 and 72 hr and at various other times indicated, six mice at each appointed time received 100 μCi ^{3}H-thymidine (sp. act. 6·7 Ci/m-mole; New England Nuclear, Boston, Mass.) i.p. and 1 hr later were sacrificed. Ascites tumor was aspirated from the peritoneal cavity with a Pasteur pipette. Additional ascites was obtained by washing the peritoneal cavity with iced phosphate-buffered 0·85% NaCl, pH 7·4 (PBS). A 3-cm length of proximal duodenum was next removed from each mouse. It was first gently agitated in iced PBS and blotted on absorbent paper to remove adherent ascitic tumor cells. It was next split lengthwise on a glass slide. A second glass slide was used to strip the mucosa from the muscularis and serosal layers. The mucosa was removed from the second slide with wooden applicator sticks and dispersed on iced PBS. The bone marrow was harvested from both tibias of each mouse by removing the tibias, stripping the muscle from the bone, sectioning the bone at each end, and expressing the marrow using a 25-gauge hypodermic needle and a syringe of iced PBS. Separate samples of ascites tumor, duodenal mucosa and bone marrow from each of six mice were pooled into two groups of three. These pooled samples were spun at 800 *g* for 5 min in a refrigerated centrifuge (4°); the supernatant was discarded and the button of tissue was frozen for further processing. The DNA of each of the pooled specimens was extracted by a modification of the Schneider method.[17] A 0·5-ml aliquot of the final supernatant was added to 15 ml Aquasol (New England Nuclear, Boston, Mass.) and counted in a Packard Tri-Carb liquid scintillation spectrometer. A 0·5-ml aliquot of the final supernatant was processed by the Burton method[18] for DNA determination. The results were then expressed

as cpm/μg of DNA and graphed as per cent of control over time. Variation in the incorporation of ^{3}H-TdR into DNA among the control groups utilized in these studies was 10–15 per cent: bone marrow, 210 $\pm$ 30 cpm/μg of DNA; gastrointestinal mucosa, 330 $\pm$ 50 cpm/μg of DNA; and ascites tumor, 12,450 $\pm$ 1460 cpm/μg of DNA.

Survival studies. BDF_1 female mice bearing L1210 ascites tumor were treated on day 6 of tumor development with BCNU, 16 mg/kg i.p. Thirty mice remained untreated and served as controls. Treated mice were subdivided into groups of 30 each, which varied only in the timing of a second (16 mg/kg i.p.) dose of BCNU. These groups were: zero-hr only (1 dose); zero-hr only (2 doses), 32 mg/kg i.p. at time zero; zero-hr and 12 hr; zero-hr and 24 hr; and zero-hr and other times as indicated in Table 1. The per cent of animals in each treatment group who survived 200 per cent longer than the animals in the untreated control group (ILS_{200}) are shown. Significance was determined using either the chi-square test or the Fischer Exact Test.

TABLE 1. SURVIVAL OF BDF_1 MICE BEARING ADVANCED L1210 LEUKEMIA* AFTER BCNU THERAPY

Treatment groups†	Per cent achieving 200% life prolongation	
1 Dose,‡ 0° only	3	
2 Doses,§ 0° only	13	$P < 0.005$
2 Doses,§ 0° + 12°	50	
2 Doses, 0° + 24°		$P < 0.05$
2 Doses, 0° + 36°	27	
2 Doses, 0° + 48°		
2 Doses, 0° + 144°		$P < 0.05$
2 Doses, 0° + 168°	48	
2 Doses, 0° + 192°		
2 Doses, 0° + 240°	27	$P < 0.05$

* Day 6 L1210 ascites tumor.
† 30 Mice in each treatment group.
‡ BCNU, 16 mg/kg i.p.
§ BCNU, 32 mg/kg i.p., total dose.

RESULTS

Alteration in DNA synthesis as reflected by the incorporation of ^{3}H-TdR into DNA in the bone marrow, gastrointestinal mucosa and ascites tumor induced in tumor-bearing mice

The simultaneous changes in DNA synthesis, as reflected by ^{3}H-TdR incorporation into DNA in the tumor (ascites) and host target tissues (bone marrow and gastrointestinal mucosa) following a single dose of BCNU of either 4, 8, 16 or 32 mg/kg i.p., are shown in Fig. 1 (A, B and C) and in Fig. 2. The results at each dose level will be discussed separately in this section. These experiments were performed to explore the possibility that differences might be observed in either the timing or the magnitude of alterations in DNA synthesis in the bone marrow, gastrointestinal mucosa or ascites tumor induced by the administration of BCNU at doses ranging from LD_0 (4 mg/kg) to LD_{50} (32 mg/kg).

BCNU, 4 mg/kg (Fig. 1A). After a single small dose of BCNU (4 mg/kg i.p.), DNA synthesis in bone marrow, as reflected by the incorporation of ^{3}H-TdR into DNA,

shows no significant depression. There is an initial rise above control levels at 1 hr, a return to control levels by 6 hr, and a period of "overshoot" of control which is initiated at 36 hr and is still greater than 200 per cent of control at 72 hr. The gastrointestinal mucosa, however, shows a depression of DNA synthesis as early as 1 hr, which reaches a nadir at 24 hr, returns to control by 48 hr and "overshoots" control levels at 72 hr. The ascites tumor also shows a suppression of DNA synthesis

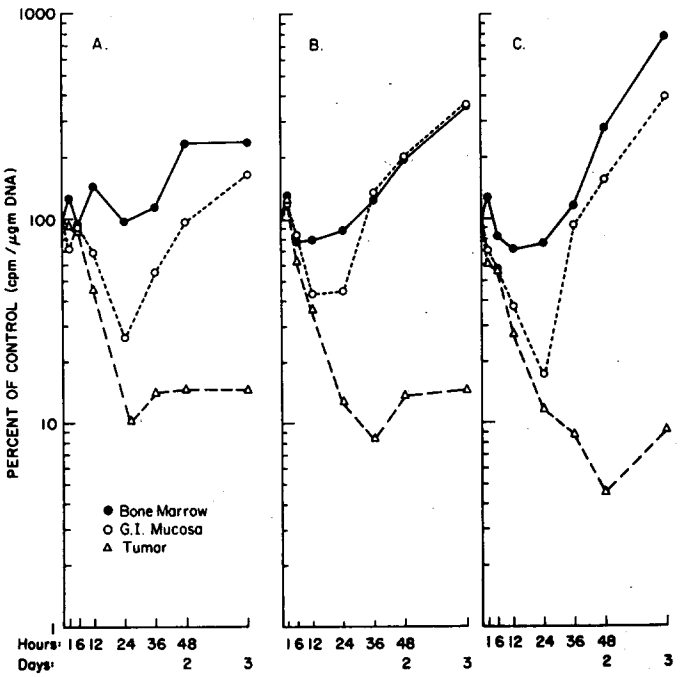

FIG. 1. Effect of a single dose of BCNU on the incorporation *in vivo* of ^{3}H-thymidine into DNA of L1210 ascites tumor, normal murine bone marrow and gastrointestinal mucosal cells: (A) BCNU, 4 mg/kg i.p.; (B) BCNU, 8 mg/kg i.p.; and (C) BCNU, 32 mg/kg i.p. Each point represents the mean of two pooled groups of three animals each. Values are expressed as the per cent of control of the cpm/μg of DNA for each of the three separate tissues. Control data (time 0°): ascites, 8400 cpm/μg of DNA; bone marrow, 200 cpm/μg of DNA; and gastrointestinal mucosa, 280 cpm/μg of DNA.

at 1 hr, which reaches a nadir at 24 hr, but there is no sign of recovery of DNA synthesis for at least the next 48 hr. At 72 hr, the incorporation of ^{3}H-TdR into DNA in the ascites tumor is still 15 per cent of control.

Even with this small dose of BCNU, marked suppression of DNA synthesis occurs in the tumor at a dose not producing any such suppression in the bone marrow. Furthermore, there are clear differences between the recovery pattern of the host target tissue and the tumor.

BCNU, 8 mg/kg (Fig. 1B). DNA synthesis in the bone marrow rises to 130 per cent of control by 1 hr, reaches a nadir of 80 per cent of control by 6 hr, returns to control levels between 24 and 36 hr, and then "overshoots" control levels for the next 48 hr. DNA synthesis in the gastrointestinal mucosa also rises above control levels at 1 hr, then falls to a nadir of 42 per cent at 12 hr, returns to control levels between 24 and 36 hr, and "overshoots" control for another 48 hr. DNA synthesis

in the ascites tumor also shows an initial rise above control levels at 1 hr and then falls to a nadir at 36 hr. DNA synthesis in the ascites tumor does not recover during the ensuing 36 hr and is only 14 per cent of control 72 hr after therapy.

Again, a discordant suppression and recovery pattern is seen between the normal target tissue and the tumor with this intermediate dose of BCNU.

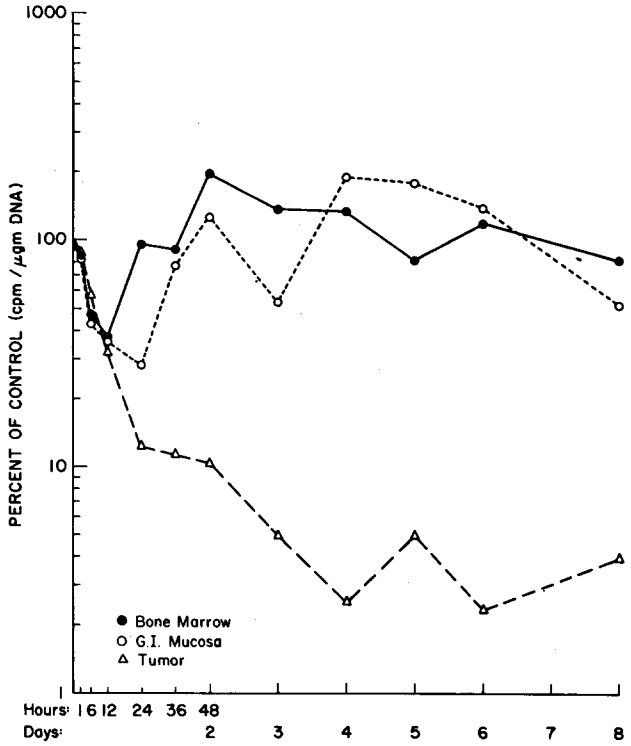

FIG. 2. Effect of a single dose of 16 mg/kg i.p. of BCNU on the incorporation *in vivo* of ^{3}H-thymidine into DNA of L1210 ascites tumor, normal murine bone marrow and gastrointestinal mucosal cells. Each point represents the mean of the two pooled groups of three animals each. Values are expressed as per cent of control of the cpm/μg of DNA of each of the three separate tissues. Control data (time 0°): ascites, 8400 cpm/μg of DNA; bone marrow, 200 cpm/μg of DNA; and gastrointestinal mucosa, 280 cpm/μg of DNA.

BCNU, 16 mg/kg (Fig. 2). After a single dose of BCNU (16 mg/kg), DNA synthesis, as reflected by the incorporation of ^{3}H-TdR into DNA, in the bone marrow was mildly suppressed by 1 hr, reached a nadir of 37 per cent of control by 12 hr, "overshot" control levels between 36 and 48 hr, and returned to baseline by the fourth and fifth day after therapy. The gastrointestinal mucosa also showed initial suppression of DNA synthesis by 1 hr after therapy, reached a nadir of 28 per cent of control by 24 hr and "overshot" control from day 4 through day 6. The ascites tumor also showed a modest decline in DNA synthesis by 1 hr after therapy. The suppression of DNA synthesis in the ascites tumor then continued to a nadir of 2·5 per cent of control by 4 days and remained suppressed through day 8, at which time DNA synthesis in the ascites tumor was 4 per cent of control.

BCNU, 32 mg/kg (Fig. 1C). After a single dose of BCNU (32 mg/kg i.p.), the incorporation of ^{3}H-TdR into DNA in the bone marrow rose to 130 per cent of control

by 1 hr after therapy; by 6 hr after therapy, DNA synthesis was depressed to 80 per cent of control and maximal suppression was achieved by 12 hr. Return to control levels of DNA synthesis occurred between 24 and 36 hr, and from 36 through 72 hr after therapy, levels of DNA synthesis in the bone marrow "overshot" control. In the gastrointestinal mucosa, DNA synthesis was initially depressed 1 hr after therapy, reached a nadir of 17 per cent of control by 24 hr, returned to control by 36 hr and "overshot" control from 36 through 72 hr after therapy. The ascites tumor also showed an initial suppression of DNA synthesis by 1 hr after therapy. Suppression of DNA synthesis continued in the ascites tumor to a nadir of 4·5 per cent of control by 48 hr and was still depressed (9 per cent of control) at 72 hr after therapy.

Thus, with stepwise increases in BCNU doses, increasing suppression of DNA synthesis occurred in all three target tissues; however, with all four doses of BCNU, the tumor was more severely suppressed and recovered more slowly than the normal tissues.

Alterations in DNA synthesis in the bone marrow and gastrointestinal tract of normal mice induced by BCNU, 32 mg/kg

To explore the possibility that a second, but delayed, suppression of DNA synthesis reflected by ^{3}H-TdR incorporation into DNA of the host target tissues (bone marrow and gastrointestinal mucosa) might occur after BCNU therapy, normal mice were treated with BCNU, 32 mg/kg i.p., and studied for 52 days (Fig. 3). DNA synthesis in the bone marrow showed a pattern of suppression and recovery as seen previously (Fig. 1C). Maximal suppression of DNA synthesis was noted by 24 hr and "overshoot" of control was seen by 48 hr after therapy. DNA synthesis in the bone marrow did not show a period of pronounced suppression during the remainder of this 52-day study.

DNA synthesis in the gastrointestinal mucosa also showed a pattern of suppression and recovery which was maximal at 48 hr and "overshot" control by 72 hr after therapy. The overshoot was followed by a return to control levels over the next 5 days. From day 9 through day 52, DNA synthesis in the gastrointestinal mucosa showed moderate fluctuations with respect to control levels. At three times after therapy, DNA synthesis in the gastrointestinal mucosa fell below control levels: day 17 (42 per cent of control), day 27 (52 per cent of control) and day 48 (72 per cent of control).

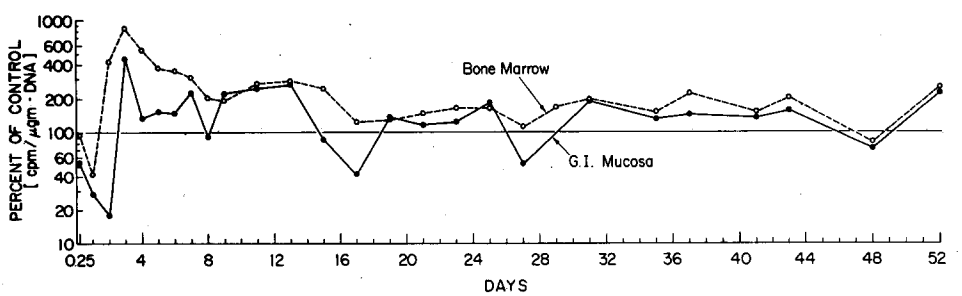

FIG. 3. Effect of a single dose of 32 mg/kg i.p. of BCNU on the incorporation *in vivo* of ^{3}H-thymidine into DNA of normal murine bone marrow and gastrointestinal mucosal cells. Each point represents the mean of two pooled groups of three animals each. Values are expressed as per cent of control of the cpm/µg of DNA for each of the two separate tissues. Control data (time 0°): bone marrow, 150 cpm/µg of DNA; and gastrointestinal mucosa, 340 cpm/µg of DNA.

Effect of dose schedule upon survival of mice with advanced L1210 leukemia

The single dose studies with BCNU have all shown discordant recovery patterns of DNA synthesis between the normal tissues and the tumor, in each instance showing more profound and persistent suppression in the tumor. This suggested that the proper timing of the second dose at a point when the tumor was beginning recovery, but normal tissues had either attained their nadir ^{3}H-TdR incorporation into DNA or had completed recovery, might result in an improved chemotherapeutic effect.

To study the effect of varying dose schedules of BCNU administration upon the survival of animals with advanced L1210 leukemia, mice bearing a 6-day growth of L1210 leukemia were initially treated with BCNU, 16 mg/kg i.p., and later received a second dose of BCNU, 16 mg/kg i.p., at a time dictated by the particular treatment group (Fig. 2). A single dose of BCNU, 16 mg/kg, at time zero only allowed 3 per cent of the mice to achieve an ILS_{200}. When the two doses of BCNU were given simultaneously at time zero (32 mg/kg), the ILS_{200} was 13 per cent ($P < 0{\cdot}005$). If a second dose of BCNU is given 12 hr after the first, 50 per cent of the mice achieve an ILS_{200} as compared to the 13 per cent seen when the entire 32 mg/kg is given at time zero ($P < 0{\cdot}005$). If a second dose of BCNU is given 24, 36 or 48 hr after first dose, the ILS_{200} is only 27 per cent as compared to the 50 per cent achieved with the zero-hr and 12-hr schedule ($P < 0{\cdot}05$). If, however, the second dose of BCNU is delayed until 144, 168 or 192 hr after the first dose, 48 per cent of the animals so treated achieve an ILS_{200} as compared to only 27 per cent when the second dose is further delayed to 240 hr after the first dose ($P < 0{\cdot}05$).

DISCUSSION

In the present studies we have demonstrated that after a dose of BCNU, a pattern of suppression and recovery of DNA synthesis, as reflected in the incorporation of ^{3}H-TdR into DNA, occurs in the host target tissues (bone marrow and gastrointestinal mucosa) which is different from that seen in the tumorous tissue studied (Figs. 1 and 2). The time course of the suppression and recovery of DNA synthesis in the bone marrow and gastrointestinal mucosa was similar. The onset of suppression of DNA synthesis in both tissues was 6 hr after BCNU treatment, maximal suppression was noted by 12 hr for the bone marrow and by 24 hr for the gastrointestinal mucosa. Recovery was complete by 36–48 hr for both tissues, and thereafter a period of "overshoot" of control levels of DNA synthesis ensued. Although the timing of suppression and recovery of DNA synthesis was similar at the four doses studied, a dose–response effect was seen to the extent that magnitude of suppression of DNA synthesis in bone marrow and gastrointestinal mucosa was greater at the two higher doses (16 and 32 mg/kg) than at the two lower doses (4 and 8 mg/kg).

DNA synthesis in the ascites tumor fell to approximately 10 per cent of control levels by 24 hr after BCNU administration at all four dose levels. A dose–response effect was again noted in that DNA synthesis fell well below 5 per cent of control at 16 and 32 mg/kg, whereas it did not reach these levels at the 8 and 4 mg/kg dosages. The effect of 16 mg/kg of BCNU on DNA synthesis in normal and tumorous tissues was studied for a prolonged period (8 days). DNA synthesis in the ascites tumor was still markedly depressed (4 per cent of control) 8 days after BCNU

therapy. By that time, DNA synthesis simultaneously studied in the host target tissues (bone marrow and gastrointestinal mucosa) had been suppressed, recovered, overshot and returned to control levels. Thus, a differential was seen between the suppression and recovery of DNA synthesis induced by a single dose of BCNU in the host target tissues as compared to the tumorous tissue (L1210 ascites).

The therapeutic index of BCNU might be improved by taking advantage of the patterns of suppression and recovery of DNA synthesis in the bone marrow and gastrointestinal mucosa. Bruce *et al.*[19,20] suggested in 1966 that cycle-specific agents such as BCNU[21] be given at high doses over a short period of time and not be repeated until the marrow function has returned to normal, as toxicity is directly dependent upon the proliferative state of the host target tissue. As the time to host target tissue recovery was not dose dependent, one might be able to reduce toxicity and thus increase the therapeutic index of BCNU by administering one-half of the total dose of the drug at time zero and then giving the second half at one of two time points: (1) at the nadir of DNA synthesis of the host target tissues, or (2) at a time when the host target tissues have recovered. To test this hypothesis, mice bearing advanced L1210 leukemia were initially treated with 16 mg/kg of BCNU and, at various points in time depending on the treatment group, received a second dose of BCNU, 16 mg/kg (Table 1). If the entire dose of BCNU was given at time zero (32 mg/kg), 13 per cent of the mice achieved an ILS_{200}. If half the dose was given at time zero and half at the nadir of DNA synthesis in the bone marrow (12 hr), a significant increase in survival was obtained ($P < 0{\cdot}005$) and the ILS_{200} rose to 50 per cent. If the second half of the BCNU dose was delayed to the period of recovery of DNA synthesis in the bone marrow (24, 36 or 48 hr), then the significant gain in survival was lost due to a probable increase in host tissue toxicity. When the second half of the dose was delayed to the period when DNA synthesis in the host tissue returned to control levels (144–192 hr), we regained the significant increase in survival ($P < 0{\cdot}005$). If longer delay occurred and the second half of the BCNU dose was administered 240 hr after the first, therapeutic advantage was once again lost, this time presumably due to excessive tumor regrowth between treatment doses. Thus, the therapeutic index of a single dose of BCNU was increased, if that dose was split and the second half administered either at the nadir of DNA synthesis in the host target tissues or soon after the return of DNA synthesis to control levels in these tissues. Therapeutic advantage was lost if one administered the second half of the dose at a time when the normal host tissues were rapidly proliferating, i.e. the recovery phase of DNA synthesis, or if prolonged delay between the two doses allowed a significant interval for tumor recovery.

A unique aspect of the toxicity seen with the nitrosoureas, which tends to becloud their mechanism of action, is exemplified by the delayed bone marrow toxicity seen in man after BCNU treatment.[16] No good animal model exists at present for this clinically important aspect of the delayed toxicity of the nitrosoureas. With 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), delayed hepatotoxicity has been seen in dogs[22] and delayed renal toxicity has been seen in monkeys.[23] We thought it might be possible to see subtle delayed changes in DNA synthesis in the bone marrow, even though the animal did not manifest delayed leukopenia in the peripheral blood. Studies of DNA synthesis, as reflected in the incorporation of ^{3}H-TdR into DNA in the bone marrow and gastrointestinal mucosa of normal mice after BCNU

(32 mg/kg i.p.) for 52 days (Fig. 3), although demonstrating several fluctuations in DNA synthesis of the gastrointestinal mucosa, failed to reveal delayed depression of DNA synthesis in the bone marrow.

The sequential studies on the alterations in DNA synthesis induced by BCNU suggested possible clues as to the mechanism of action of the drug. After 4, 8 or 32 mg/kg of BCNU (Fig. 1, A–C), an initial increase in the incorporation of ^{3}H-TdR was observed in several of the tissues studied. It is unlikely that this early change represents a true increase in total DNA synthesis, but rather probably a decrease in the *de novo* thymidine pool with resultant preferential incorporation of preformed thymidine. DNA synthesis in both the normal and tumorous tissues did show initial depression by 6 hr after BCNU administration at all four doses, with the exception of the bone marrow at the LD_0 dose of 4 mg/kg. A slightly longer delay in the onset of depression of DNA synthesis induced by BCNU in L1210 solid tumor was noted by Wheeler and Bowden,[11] in which the initial depression of DNA synthesis was not noted until 16 hr after BCNU administration. The two studies differ in several respects which might account for variances in the observations: (1) our studies utilized i.p. L1210 ascites tumor, while Wheeler and Bowden[11] used subcutaneously implanted L1210 solid tumor; (2) our studies employed ^{3}H-TdR, a preformed pyrimidine precursor, whereas Wheeler and Bowden used adenine-8-^{14}C, a purine precursor; and (3) although similar doses of BCNU were utilized, since the route of administration was i.p. in both studies, the i.p. ascites tumor might be exposed to a greater concentration of the drug than a subcutaneous tumor.

It is possible then that BCNU might interfere with both purine and pyrimidine biosynthesis, which would be reflected in a decrease in their respective *de novo* pool sizes and could explain the early increase in incorporation of preformed purine (adenine-8-^{14}C) into DNA seen and suggested by Wheeler and Bowden[11] and the increase in incorporation of preformed pyrimidine into DNA (^{3}H-TdR) seen in the present studies after BCNU administration. If BCNU interferes with *de novo* purine synthesis, one would expect to see the incorporation of ^{14}C-formate into DNA-adenine (^{14}C) reduced at a time when the incorporation of adenine-8-^{14}C was increased. Wheeler and Bowden[11] found that 8 hr after BCNU therapy ^{14}C-formate incorporation into DNA-adenine (^{14}C) is approximately 70 per cent of control, while incorporation of preformed adenine-8-^{14}C into DNA is approximately 145 per cent of control. They also observed an early rise (4 hr after BCNU therapy) of formate-^{14}C incorporation into DNA-adenine-^{14}C. Since formate is a methyl donor for both *de novo* purine and pyrimidine synthesis,[24,25] if *de novo* pyrimidine synthesis were interfered with prior to a block in *de novo* purine synthesis (^{3}H-TdR incorporation into DNA rose above control levels 1 hr after BCNU therapy in the present studies), more formate would be available for *de novo* purine synthesis, and thus the incorporation of formate-^{14}C into DNA-adenine-^{14}C would be expected to rise. Between 6 and 16 hr after BCNU therapy, depending on the system studied, there appears to be a more direct interference with DNA synthesis as reflected by the suppression of ^{3}H-TdR incorporation into DNA, which falls below control levels. Similar observations have been made before in studies utilizing adenine-8-^{14}C.[11]

Since the biologic half-life of BCNU is less than 1 hr[26] and the depression of DNA synthesis does not begin until at least 6 hr after BCNU therapy, it is unlikely (1) that intact molecule represents the "active" form of the drug, and (2) that the major

mechanism of action involves the direct enzymatic blockade of DNA synthesis; but rather that a metabolite such as 2-chloroethyl isocyanate might be active via carbamylation[13] or alkylation with or without significant reduction of DNA nucleotidyltransferase activity.[10]

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