Effects of Adriamycin on DNA Synthesis in Mouse and Rat Heart

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ABSTRACT

Adriamycin induces an inhibition of DNA synthesis in mouse tissues within one hr after treatment. While the effects are short-lived in liver and small intestine, DNA synthesis in heart remains below control values for up to 7 days. After this period DNA synthesis in hearts of treated mice is elevated and remains above control values for as long as 4 weeks. Both 1-β-d-arabinofuranosylcytosine and actinomycin D also induce inhibition of cardiac DNA synthesis soon after treatment; the effects of 1-β-d-arabinofuranosylcytosine are over by the end of 24 hr while the effects of actinomycin D persist for at least 4 days. Actinomycin D treatment also induces an “overshoot” of DNA synthesis in mouse heart. Adriamycin can induce a loss of prelabeled DNA from heart, although no pathological alterations are immediately obvious. The small intestine, however, shows extensive karyorrhexis. The initial effects on cardiac DNA synthesis occur in adrenalectomized animals, indicating that the effects are not mediated via the adrenal gland. We did find, however, that DNA synthesis in heart was sensitive to the effects of starvation. The results of this study indicate that inhibition of mouse heart DNA synthesis is not specific for Adriamycin and that the effects of Adriamycin in heart following a single treatment are long-lived.

INTRODUCTION

Adriamycin is an antitumor antibiotic of the anthracycline class that has induced significant responses in a wide range of solid tumors as well as in malignant lymphomas and acute leukemias (2). This antibiotic displays side effects common to many anthracycline drugs (e.g., bone marrow depression, stomatitis, alopecia), but a major dose-limiting side effect is cardiotoxicity, which may lead to fatal congestive heart failure. This effect occurs in patients in whom the total dose has exceeded 550 mg/sq m and can occur long after treatment has ended (2). Similar pathological changes have been produced in rabbits treated with Adriamycin (12). In addition, Adriamycin and daunomycin can induce ultrastructural changes in myocardial cells of rats and mice (3, 20).

Cardiac DNA synthesis in mice is inhibited soon after treatment with Adriamycin but not in animals receiving 5-fluorouracil. It has been proposed that the inhibition may be a unique proximate action of anthracycline antitumor agents that leads to the development of myocardial pathology and to death (20). We undertook to confirm and extend this observation because of its possible significance for understanding the pathogenesis of anthracycline-induced myocardial lesions. Our specific aims were to study the extent and duration of the inhibition caused by Adriamycin and to determine whether cardiac DNA synthesis was, in fact, unaffected by other antitumor agents. For the latter purpose we chose actinomycin D since, like Adriamycin, it binds tightly to DNA, and ara-C (4) since its mechanism of action is different from that of the anthracyclines, 5-fluorouracil, or actinomycin D. Because cardiac DNA synthesis is inhibited by glucocorticoids (14), we also questioned whether the inhibition caused by Adriamycin is a direct effect of one mediated secondarily through release of adrenocortical hormones as a result of drug-induced intoxication. Finally, we were interested to determine whether the doses of Adriamycin used in previous work (20) caused histopathological changes in mouse heart sufficient to account for the death of treated animals or whether there were lesions at other sites of greater significance in the lethal outcome.

MATERIALS AND METHODS

Weanling male CD-1 mice and male Sprague-Dawley rats (CD line) were purchased from Charles River Breeding Laboratories, Wilmington, Mass., and allowed food and water ad libitum. The animals were used when 4 to 5 weeks old. [Methyl-3H]thymidine (2 Ci/mm) and [methyl-14C]-thymidine (57.1 mCi/mm) were purchased from New England Nuclear, Boston, Mass. Adriamycin was supplied by Adria Laboratories Inc., Wilmington, Del.; ara-C was supplied by The Upjohn Co., Kalamazoo, Mich.; and actinomycin D was supplied by Merck, Sharp and Dohme, West Point, Pa. All solutions for injection were made in 0.9% NaCl solution and injected parenterally in a volume of 10 ml/kg as noted in the text. Animals serving as controls received injections of 0.9% NaCl solution.

Previously reported methods were used for extraction of DNA by the Schmidt-Thannhauser procedure, measurement of total DNA content, and determination of the incorporation of radiolabeled thymidine (22). In the experiments in which individual organs from single animals or pools from 2 mice were analyzed, hot 10% perchloric acid was used for the final extraction of DNA, and the absorbance of the extracts was measured at 260 nm with a Beckman DU spectrophotometer. In the remaining experiments with the use of pooled organs from 3 mice, DNA was extracted with hot 10% trichloroacetic acid, and the extracts were analyzed colorimetrically with the use of the diphenylamine.
reaction. With both methods of extraction, DNA content was expressed in \( \mu M \) equivalents of deoxyribose. For assessment of the amount of thymidine incorporated into heart mitochondrial DNA, the nuclear and mitochondrial fractions were separated by differential centrifugation. The hearts were homogenized in a solution containing 0.3 M sucrose, 0.005 M EDTA, and heparin (50 units/ml) (7). The homogenate was centrifuged at 1000 \( \times \) g for 10 min to yield the nuclear pellet. The resulting supernatant was centrifuged at 11,000 \( \times \) g for 20 min to yield the mitochondrial pellet. The cytochrome oxidase activity was determined spectrophotometrically (5). For microscopic study tissues were fixed in Bouin’s solution; sections were stained with hematoxylin and eosin.

The total radioactivity in cold acid-soluble materials and in TMP, TDP, and TTP fractions from organs of mice given labeled thymidine was determined as follows. Tissue homogenates were prepared in cold 10% trichloroacetic acid. Cold acid-soluble fractions were extracted with successive portions of ether to remove trichloroacetic acid and then dried in a rotary evaporator. The residues were redissolved in water, and portions were used for determination of total acid-soluble radioactivity and for chromatography on paper with samples of TMP, TDP, and TTP (Whatman no. 1, descending, developed with isobutyric acid:H,0:concetrated NH,OH, 66:33:1 (v/v)). The zones corresponding to the thymidine nucleotides were eluted with 0.1 N HCl for measurement of radioactivity.

RESULTS

Toxicity. In preliminary tests various doses of Adriamycin (7.5 to 30 mg/kg) were injected i.v. in groups averaging 12 mice each that were then observed for 28 days. The median lethal dose was 18 mg/kg. All mice receiving 30 mg/kg died between 4 and 9 days after injection. A dose of 20 mg/kg killed most mice; they died between 6 and 15 days. Another group of 11 mice was given 20 mg/kg i.v. and killed in pairs at 5 hr, 24 hr, and 5 days after injection for histological study by light microscopy of heart, liver, duodenum, spleen, thymus, and sternal bone marrow; the remaining 5 mice died between the third and fifth days. Steady weight loss was prominent in all; in the 2 killed at 5 days, the losses were 28 and 32% of initial weight. In the 5-hr mice extensive karyorrhexis was present in the epithelium of all duodenal crypts, and the splenic red pulp was markedly decreased. At 24 hr after injection, nuclei remaining in crypt epithelium of the duodenum were swollen and had lost polarity; a few were still karyorrhectic. Thymi had scattered karyorrhectic foci, the splenic red pulp was still reduced in amount, and there was a slight diminution of cells in the sternal bone marrow. The duodenum of the 5-day mice had flattened villi, and the mucosa consisted mainly of regenerative crypts containing uniformly and markedly basophilic epithelium with frequent mitoses. Debris was present in glandular lumina. Thymi were involuted, and both white and red pulp were severely contracted in spleens. Sternal bone marrow appeared normal. No lesions were found in either heart or liver of any of the above mice.

A large number of mice received a single dose (15 mg/kg) for study of animals that survive the acute lethal effects of Adriamycin. Chart 1, describing their fate during the first 8 weeks after injection, shows the steady weight loss that occurs during the first week and the clustering of deaths at the end of the first week. By 28 days 39% of the animals were dead. Following the first week weight recovers in survivors until about 5 to 6 weeks after injection, when a second phase of loss begins in most animals. The mice so affected die in a severely cachectic state at scattered times between 10 and 20 weeks after injection. The second wave of intoxication resembles that previously described in rats (17).

Preliminary tests of the toxicity of actinomycin D were also carried out. Groups of mice (averaging 8/dose) were given injections i.v. of varying amounts of drug (0.38 to 1.5 mg/kg) and were observed for 28 days. The median lethal dose was found to be 1.0 mg/kg; most deaths occurred 1 and 2 days after injection, and the latest occurred at 9 days. The dose of ara-C (100 mg/kg) that was used in the experiments to be described below induced no overt signs of toxicity.

Distribution of [methyl-\(^3\)H]Thymidine Incorporation into DNA of Mouse Organs. The data in Chart 2 show the incorporation of [methyl-\(^3\)H]thymidine into the DNA of mouse organs. There was marked variability in both heart and liver in contrast to the relatively uniform results obtained in small intestine, spleen, and thymus. Because of this variability in all subsequent studies we obtained control values at each time interval investigated. Chart 2 also shows that, in contrast to small intestine, spleen, and thymus, the incorporation of the precursor is essentially over by 20 min in heart and liver. Nevertheless, we chose 20-min pulse times for the following experiments to obtain sufficient radioactivity in heart and liver DNA for accurate measurement. It is, therefore, possible that inhibitions of thymidine incorporation in heart and liver may have been greater than determined.

The specific activity of mitochondrial DNA is greater than...
that of nuclear DNA in rat tissues (11). Since the extent of nuclear DNA synthesis in heart might be negligible, we determined the percentage of thymidine incorporated into nuclei and mitochondria of mouse heart with the use of cytochrome oxidase activity as a marker for the mitochondrial fraction. The mitochondrial fractions obtained in our procedure had 70% of the total cytochrome oxidase activity. The same fractions contained only 1% of the total incorporated radioactivity. It appears that essentially all of the thymidine-labeled DNA in mouse heart is of nuclear origin.

**Effects of Adriamycin on DNA Synthesis.** The data in Chart 3 indicate that Adriamycin (20 mg/kg i.v.) induced marked and rapid inhibition of DNA synthesis in heart, liver, and small intestine. In each of the 3 organs, DNA synthesis was inhibited 70 to 80% and remained at that level for at least 5 hr. Spleens and thymus were also studied in the animals shown in Chart 3. By 5 hr after injection, DNA synthesis in spleen was inhibited by 75% and in thymus it was inhibited by 60%.

Since most mice receiving Adriamycin at a dose of 20 mg/kg die within less than 2 weeks, it was necessary to use a lower dose to study the duration of inhibition of DNA synthesis. The data in Chart 4 show the effects of Adriamycin (10 mg/kg) through 14 days after treatment. Whereas synthesis in heart was still markedly inhibited at 4 days, at this time DNA synthesis in liver had returned to control levels; synthesis in small intestine showed an overshoot, suggesting regenerative activity after early injury. By 7 days thymidine incorporation in intestine was normal. The results also indicate that regenerative activity may also have occurred in hearts, since DNA synthesis in the organ was greater than that of controls at 14 days after treatment. To determine the maximum overshoot in heart and its duration, we increased the dose of Adriamycin to 15 mg/kg, about the highest possible dose permitting sufficient numbers of mice to survive for 4 to 5 months; this dose kills 39% of treated animals within the first 4 weeks after injection (see above). As shown in Chart 5, the increased dose did result in greater inhibition at 7 days after injection than in the mice of Chart 4 receiving 10 mg/kg. The overshoot observed at 2 weeks after treatment was more than double the control values, and it remained significantly greater than that of controls even as late as 4 weeks. At 6 weeks after injection, DNA synthesis returned to control levels.

It is assumed above that inhibitions of thymidine incor-

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**Chart 2.** Incorporation of [methyl-3H]thymidine into DNA of mouse organs. Two experiments were done. In the first 4-week-old mice were given [methyl-3H]thymidine (500 µCi/2 µmol/kg s.c.) at 0 time and killed in pairs at various times thereafter. The hearts, livers, small intestines, spleens, and thymuses from each pair were pooled. The results obtained with each pool is plotted as a single point. The results for small intestine and spleen were multiplied by 0.25 before plotting in the chart. In the second experiment 5-week-old mice were given 1000 µCi/0.5 µmol/kg s.c. and were killed in groups of 3. The hearts and livers were pooled from each group. The results obtained from these pools were multiplied by 0.5 and plotted as the encircled points. To obtain the actual specific activity (Sp. Act.) one must multiply the numbers shown on the ordinate by 2000 for the encircled points for heart and liver, by 4000 for small intestine and spleen, and by 1000 for the remaining data for heart, liver, and thymus.

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**Chart 3.** The effect of Adriamycin (20 mg/kg i.v.) on DNA synthesis in mouse tissues. At various intervals after 4-week-old mice had been given 0.9% NaCl solution (O) or Adriamycin (•), the animals were given [methyl-3H]thymidine (500 µCi/2 µmol/kg s.c.), and they were killed 20 min later. Results obtained from each mouse are shown as individual points plotted at the time of killing. To obtain the actual specific activity (Sp. Act.), one must multiply the numbers on the ordinate for heart and liver by 1000. The data for small intestine, multiplied by 0.25 for plotting, are to be multiplied by 4000.

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**Chart 4.** The effect of Adriamycin (10 mg/kg i.v.) on DNA synthesis in mouse tissues. At various intervals after giving 4-week-old mice either 0.9% NaCl solution (O) or Adriamycin (•), the animals were given [methyl-3H]thymidine (500 µCi/2 µmol/kg s.c.), and they were killed 20 min later. The data are given as means ± S.D. (n = 4). To obtain the actual specific activity (Sp. Act.), one must multiply the numbers on the ordinate for heart and liver by 1000. The means ± S.D. for small intestine, multiplied by 0.125 for plotting, are to be multiplied by 8000.
poration reflect real inhibitions of DNA synthesis. This is reasonable if there are no disturbances caused by the drug in the tissue uptake and phosphorylation of exogenous thymidine. In 1 experiment we looked for such disturbances and found none. For this purpose we gave mice Adriamycin (10 mg/kg) or 0.9% NaCl solution i.v., and 3 hr later we gave labeled thymidine (1000 μCi/kg) s.c. Ten min thereafter the mice were killed, and the hearts, liver, and small intestines from 3 animals were pooled.

In the treated animals, by comparison with controls, the incorporation of the labeled thymidine into the DNA of heart, liver, and intestine was inhibited by 50, 43, and 45%, respectively. The total cold acid-soluble radioactivities in each organ pool from control and treated mice were, respectively (in units of 10^6 dpm), 2.27 and 2.33 in hearts, 106 and 103 in livers, and 33.7 and 32.2 in intestines. The total radioactivities recovered in TTP fractions of control and treated pools were, respectively (in units of 10^3 dpm), 18 and 17 in hearts, 480 and 560 in livers, and 200 and 222 in intestines. Analyses of the radioactivities in the TMP and TDP fractions similarly revealed no appreciable differences between controls and treated tissues.

**Effects of Actinomycin D and ara-C on DNA Synthesis.** The results in Table 1 show that in heart and small intestine ara-C inhibited DNA synthesis by more than 95% within 1 hr. In comparison with that of Adriamycin, this effect is of short duration; at 1 day the specific activity of heart DNA in ara-C-treated mice was equal to that of control, while in small intestine there appeared to be an overshoot. [methyl-^3H]Thymidine incorporation into heart DNA of mice treated with actinomycin D was inhibited at 5 hr, markedly inhibited at 1 day, and had not fully returned to control values at 4 days, a result similar to that obtained in mice given Adriamycin. In small intestine, DNA synthesis was markedly inhibited by actinomycin D at 1 day and returned to control values by the fourth day.

It was of interest to determine whether ara-C and actinomycin D induce a delayed overshoot in DNA synthesis in heart like that found in mice at 2 and 4 weeks after injection of Adriamycin (Chart 5). For this purpose mice were given ara-C (100 mg/kg) or actinomycin D (0.75 mg/kg), and DNA synthesis was measured 2 weeks later, as described in Chart 5. In the ara-C experiment the mean specific activities (10^3 dpm/μmol deoxyribose) of control and treated mice were, respectively 8.6 and 9.9; with actinomycin D the mean activities in controls and treated mice were, respectively, 10.6 and 27.7. The increase in DNA synthesis in the actinomycin D-treated mice was as great as that observed in the Adriamycin-treated animals shown in Chart 5.

**Adriamycin-induced Loss of Prelabeled DNA.** The cytostatic effects of antitumor agents are associated with accelerated losses of thymidine-labeled DNA (18). To determine whether Adriamycin might have similar effects, we measured the loss of prelabeled DNA induced by the agent in mouse organs (Chart 6). The amount of [methyl-^3H]thymidine incorporated into DNA following a 1-hr pulse is expressed as 100%. Control mice, receiving 0.9% NaCl solution at the end of the 1-hr pulse, lost about 20% of the prelabeled DNA in both heart and liver within the first day and 30 to 40% by Day 4. At Day 7 (not shown), the amount of radioactive DNA remaining was similar to that at Day 4 (see "Discussion" for the significance of the 24-hr losses in

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*a To obtain the actual specific activity, one must multiply the data by 1000. Each value represents data from a pool of 3 animals.

**Table 1**

The effect of actinomycin D and ara-C on DNA synthesis in mouse heart and small intestine

At various intervals after 5-wk-old mice had been given either 0.9% NaCl solution (controls), actinomycin D (0.75 mg/kg), or ara-C (100 mg/kg) i.v., the animals were given [methyl-^3H]thymidine (1000 μCi/0.5 μmol/kg s.c.), and they were killed 20 min later at the times indicated.

Chart 5. The effect of Adriamycin (15 mg/kg i.v.) on DNA synthesis in mouse heart. At various intervals after 5-week-old mice had been given either 0.9% NaCl solution (O) or Adriamycin (•), the animals were given [methyl-^3H]thymidine (1000 μCi/0.5 μmol/kg s.c.), and they were killed 20 min later. At each interval the hearts were pooled in groups of 3. Each point represents the mean ± S.D. for 4 heart pools. To obtain the actual specific activity (Sp. Act.), one must multiply the numbers on the ordinate by 1000.
controls). In small intestine there was no loss of total counts until 36 hr (calculated), a finding consistent with that obtained by others (15). In Adriamycin-treated mice the losses of total counts in each organ were greater than those seen in controls. In intestine the differences were significant at 1, 2, and 4 days (p < 0.01). By Day 4 the losses in heart and liver were 70%, a value significantly greater than that in controls (p < 0.01). In mice treated with ara-C compared with controls, significantly greater losses of labeled DNA occurred in heart and small intestine at 1 and 2 days (p < 0.01). The ara-C effects in liver were less dramatic and more variable; at Day 2 the variability resulted in large standard deviations and the mean was similar to that of controls.

We also studied the loss of prelabeled DNA from heart, liver, and small intestine of mice treated with actinomycin D (0.75 mg/kg). The results at 1 and 2 days after treatment were similar to those seen following Adriamycin. The percentages of total dpm in heart at 1 and 2 days after actinomycin D were 65 and 51, respectively; in liver they were 43 and 54, respectively; and in small intestine they were 73 and 64, respectively.

Since it is conceivable that the greater loss of prelabeled DNA was due to an effect of Adriamycin in combination with alterations induced by the radioactivity associated with tritiated thymidine used as a precursor for DNA synthesis, we determined the loss of DNA prelabeled with [methyl-14C]thymidine at a level of 0.1 mCi/kg. The results were similar to those obtained with the use of the higher dose of tritiated precursor.

**Indirect Effects of Adriamycin on DNA Synthesis.** It was conceivable that the prolonged decreases in DNA synthesis in hearts and livers noted in Chart 4 as late as 4 days after the administration of Adriamycin might have been due, in part, to general toxic effects and the lack of weight gain resulting therefrom. We determined, therefore, DNA synthesis in heart, liver, and small intestine of mice that had been deprived of food for 24 hr and had lost 18% of their initial body weight, an amount comparable to losses sustained by mice within the first 4 days after receiving single doses of Adriamycin and actinomycin D like those used in the above studies. The data in Table 2 show that DNA synthesis in heart and liver was in fact reduced in starved animals to an extent similar to that seen in mice at 4 days after drug treatment (Chart 4). The small intestine was less affected.

Because it is known that adrenocortical hormones can inhibit DNA synthesis in rat heart and liver but not in small intestine (14), it was possible that the acute inhibitory effects of Adriamycin on the 2 former organs were also due to indirect effects such as the release of adrenal steroids as the result of the stressful actions of the drug. Table 3 shows, however, that the inhibitory effects of Adriamycin on [methyl-3H]thymidine incorporation into the DNA of heart, liver, and small intestine are similar in both intact and adrenalectomized rats. At 5 hr after Adriamycin, the inhibitions in heart, liver, and small intestine were approximately 90, 75, and 94%, respectively. It is curious that DNA synthesis was greater in hearts of adrenalectomized rats than in intact rats. No such difference was evident in liver or small intestine.

**DISCUSSION**

This work has confirmed earlier studies (1, 20) that showed that the incorporation of thymidine into the DNA of mouse heart is inhibited by Adriamycin; we conclude, therefore, that the drug can inhibit cardiac DNA synthesis. This is consistent with the proposal that RNA and DNA syntheses are inhibited by Adriamycin as the result of its binding to DNA (6). (Alternatively, there is a remote possibility that Adriamycin induces increases in de novo thymidylate pools sufficiently to dilute exogenous thymidine and, thus, to result in a simulated inhibition of incorporation into
DNA. At present there is no published evidence that the drug can so affect thymidylate pools.

We have also found that, in mice in which cardiac DNA is prelabeled by injection of tritiated thymidine, Adriamycin causes a significant loss of labeled DNA, a result suggesting cytotoxicity in some component(s) of the myocardium. The myocardial component(s) sensitive to inhibition of DNA synthesis by Adriamycin, ara-C, and actinomycin D is probably capillary endothelium or connective tissue cells, or both, for early in postfetal life, proliferation ceases in myocytes although it persists thereafter in stromal components (16).

Previous workers have proposed that the inhibition of cardiac DNA synthesis may be a specific effect of Adriamycin and possibly may be related to its cardiotoxic effects (20). It has been further suggested that measurement of DNA synthesis might, therefore, serve as a useful bioassay for the selection of anthracycline derivatives with less cardiotoxic potential than Adriamycin. Support for the specificity of the effect comes from the observation that another antitumor agent, 5-fluorouracil, does not inhibit DNA synthesis in mouse heart (20). Our present results, however, do not support the specific nature of the Adriamycin inhibition and its correlation with cardiotoxicity, since we have found that actinomycin D and ara-C also inhibit DNA synthesis in mouse heart and also induce losses of prelabeled DNA from this organ. As far as we know, neither ara-C nor actinomycin D has been implicated as an agent with the capacity to induce cardiac lesions in laboratory animals or man.

We also differ with a previous suggestion that the acute lethal actions of Adriamycin in mice (i.e., those causing death within the first 2 weeks after injection of the drug) may be the direct consequence of cardiac damage (20). The lesions induced during this period in intestine are more severe and more impressive than those in the heart. In fact, we have been unable to detect significant microscopic lesions in hearts of mice receiving acutely lethal doses. Presumably, the intestinal lesions are a major factor in acute fatality. In addition, nonlethal doses of actinomycin D and ara-C can induce inhibition of cardiac DNA synthesis equal to or greater than that caused by Adriamycin.

Increased proliferative activity has been observed in endothelial cells of rabbit hearts that have been X-radiated (10). This begins several weeks after irradiation and persists for several months. Since the increased proliferation coexists with pathological changes in nondividing endothelial cells, it is believed to be a compensatory mechanism of cell renewal in response to radiation-induced injury. The response is, however, inadequate and eventually capillary loss leads to ischemia and myocardial fibrosis (9). The overshoot of DNA synthesis in heart observed at 2 weeks after treatment with Adriamycin (Chart 5) may also represent the proliferation of endothelial or other interstitial cells to replace those acutely killed by the drug; this process continues for several weeks. However, its relationship to the myocardial fibrosis that is induced by chronic treatment with Adriamycin is not clear. Although rapidly developing necrosis of endothelial cells has been seen in hearts of rats (3) and rabbits (17) given high single doses of daunomycin and Adriamycin, respectively, endothelial cell necrosis that is delayed in onset and that persists for many weeks has not been observed in the hearts of rabbits treated chronically with Adriamycin (9). Moreover, actinomycin D also induces such an overshoot without subsequently causing an Adriamycin-like cardiomyopathy. Chronic treatment of animals with Adriamycin during overshoot periods, when cells could conceivably be more susceptible to an agent that interacts with DNA (6), did not result in an incidence of cardiac lesions greater than that observed in animals receiving single doses (13). It must be considered that the myocardial fibrosis caused by Adriamycin is in all likelihood a direct effect on the myocytes (9), and it remains unclear why the effect develops long after treatment has ended.

Since Adriamycin treatment can result in severe intoxication, manifested by large losses of weight, it is important to consider whether effects on DNA synthesis are due to Adriamycin directly or to adrenocortical hormones released by stress. Others have shown that adrenocorticoids can inhibit thymidine incorporation into the DNA of heart, liver, and other organs (14). Our data clearly indicate that the early inhibition of DNA synthesis observed during the first few hr after Adriamycin injection is not due to release of adrenocortical hormones. However, the persisting inhibition that is seen as late as 4 days after treatment, when animals are ill, may, at least in part, be secondary to the stress of general intoxication.

We have assumed that accelerated losses of prelabeled DNA are evidence for cell death in tissues; this is in keeping with the widely shared view holding DNA to be a stable molecule that does not turn over in mammalian tissues in the absence of cell loss. Cleaver (4) has marshaled the
evidence in favor of the orthodox view, although he admits that it remains an assumption. A contrary opinion holds to the belief that there is a form of DNA with a rate of turnover more rapid than that of cell turnover. Much of the evidence for this view comes from observations of early losses of labeled DNA that occur at rates seemingly in excess of proliferative activity. The 30 to 40% loss of label during the first 4 days in liver and heart of the control mice shown in Chart 6 may be an example. Thus, the magnitude of the loss in untreated controls cannot be explained at present by attributing it to cell renewal of capillary endothelial cells, for which turnover times have been estimated to be as long as several months (8, 19). Until other stromal components can be identified in heart with sufficiently rapid turnover to account for the control data of Chart 6, some doubt must remain concerning the proper explanation of the accelerated losses of prelabeled DNA that are caused by Adriamycin, actinomycin D, and ara-C.

ACKNOWLEDGMENTS

The authors thank Dr. Hans Marquardt for many helpful suggestions and discussions and express their appreciation to Pedro Vidal for excellent technical assistance.

REFERENCES

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