Biochemical Studies with the Vinca Alkaloids
I. Effect on Nucleic Acid Formation by Isolated Cell Suspensions

J. F. RICHARDS, R. G. W. JONES,1 AND C. T. BEER2

Cancer Research Centre and Department of Biochemistry, University of British Columbia, Vancouver, Canada

Summary

The incorporation of sodium formate-14C into nucleic acids by suspensions of rat thymus cells was inhibited in the presence of vinblastine or vinleurosine. Vincristine produced a lesser effect. The effects of vinblastine on the thymus cell system in vitro are compared with the action of the drug on thymus and other tissues under a variety of experimental conditions. The results indicate that the action of the alkaloid in the thymus system is related to its biologic effect on bone marrow and lymphoid tissue in intact animals. Both in vitro and in vivo, the incorporation of sodium formate-14C into DNA was inhibited to a greater degree than the incorporation into RNA in sensitive tissues. There is a differential effect on labeling of the purines and thymine of DNA. In cells incubated with vinblastine, the specific activities of the DNA-purines are decreased, relative to control values, earlier and to a greater extent, than is that of DNA-thymine.

Introduction

The chemical, biologic, and clinical properties of the group of oncolytic agents, the Vinca alkaloids, have been summarized in a recent review (10). More than 50 alkaloids have been isolated from the plant V. rosea Linn. (10), but only 4 of these possess antitumor activity. Vinblastine and vincristine were studied experimentally and are currently being used in the treatment of human malignant disease. Two other alkaloids, vinleurosine and vinrosidine, have been studied mainly with animal tumors. Very few studies of the biochemical action of these compounds have been reported, and the majority of these reports have been concerned only with vinblastine (VLB). As yet, the biologic functions of VLB, such as antitumor action (10, 11, 15), depression of bone marrow in rats (16) and humans (10), the involution of the thymus gland in rats (15), and the arrest of cell division in metaphase in animals (3, 7) and in tissue culture (11, 17), cannot be explained in biochemical terms. Present evidence suggests that VLB does not interfere with energy-yielding reactions in animal tissues. The oxygen uptake of liver cells from rats treated with VLB was not different from that of cells from untreated control animals (2), nor were the respiration and glycolytic functions of VLB, such as antitumor action (10, 11, 15), depression of bone marrow in rats (16) and humans (10), the involution of the thymus gland in rats (15), and the arrest of cell division in metaphase in animals (3, 7) and in tissue culture (11, 17), cannot be explained in biochemical terms. Present evidence suggests that VLB does not interfere with energy-yielding reactions in animal tissues. The oxygen uptake of liver cells from rats treated with VLB was not different from that of cells from untreated control animals (2), nor were the respiration and glycolysis of S-180 ascites cells affected by the addition of VLB (10). It has been reported that VLB increased acid production and inhibited the aerobic respiration of a thioguanine-resistant strain of L1210 leukemic cells (9). This may not be a specific effect, since other workers (12) have reported an increased lactate production by human leukemic cells in vitro in response to diazauricil, 8-azaguanine, 6-mercaptopurine, triethylene thiophosphoramide, or VLB.

Since VLB interferes with cell division, there should at some time be a demonstrable effect on nucleic acid formation, particularly of DNA, by sensitive tissues, even if this is not the primary site of action of the alkaloid. Evidence on this point is somewhat conflicting. The incorporation of radioactive precursors into nucleic acids and proteins by S-180 ascites cells in vitro was not inhibited by the addition of VLB or vincristine (10). A more recent report (6) indicated that the synthesis of soluble RNA (sRNA) was inhibited in Ehrlich ascites cells in treated animals, although synthesis of DNA was not affected at the same dose. With this tumor in vitro, incorporation of labeled precursors into DNA and the total RNA fractions was inhibited only by very high concentrations of alkaloid (6).

Evidence of an interference with DNA synthesis in cells exposed to VLB has been reported in other publications. The treatment of an ascitic lymphoma in tissue culture with VLB produced a decrease in the amount of DNA between 6 and 96 hr. The amount of RNA was not different from that of control cells (22). Experiments in vivo with rats (2) demonstrated that the incorporation of sodium formate-14C into the nucleic acids of certain tissues, particularly bone marrow, was markedly reduced by the administration of VLB. This process was not affected in all rapidly dividing tissues, suggesting a selectivity of action of VLB (2).

This paper reports some experiments on the biochemical effects of the Vinca alkaloids in vitro. Because of the marked sensitivity of rat bone marrow to VLB as measured biologically (16) and by the incorporation of isotope into nucleic acids (2), this tissue was chosen for the initial studies. However, some nucleic acid derivatives occur in tissues in very low concentrations, and since rat bone marrow is not readily obtainable in large amounts, suspensions of rat thymus cells have been used in most of this work. In whole animals, large doses of VLB have produced thymic involution (15), and a fall in circulating lymphocytes is part of the response to smaller, nontoxic amounts of the alkaloid (16). In vitro, isotope incorporation into nucleic acids by rat bone marrow or thymus cells is equally sensitive to the addition of VLB.

1 Present address: Microbiology Unit, Department of Biochemistry, Oxford University, England.
2 Medical Research Associate, Medical Research Council of Canada.

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Preparation of Cell Suspensions

DNA of thymus cells. The incubation system is described in the text.

Materials and Methods

Preparation of Cell Suspensions

ANIMALS. (a) Rats: Female rats of the Wistar strain were used as a source of bone marrow and thymus cells. Bone marrow was obtained from animals weighing 200 ± 10 gm. Thymus glands were removed from younger rats of the same strain, weighing 120 ± 10 gm. The chloroleukemia was maintained in Sprague-Dawley rats. All animals had free access to water and Purina laboratory chow. (b) Mice: The Ehrlich ascites tumor was maintained by serial transplantation in Swiss mice.

BONE MARROW SUSPENSIONS. The femurs were removed from animals following light ether anesthesia and decapitation. The marrow was ejected by centrifugation from bisected bones into chilled Robinson's medium containing glucose, 0.1 M (19). The marrow cells were resuspended in fresh medium and centrifuged for 6 min at 0°C, 1000 X g. The packed cells were resuspended in fresh medium, and aliquots of the suspension were added to the incubation vessels. The yield of tissue was approximately 80 mg wet weight/animal.

THYMUS CELL SUSPENSIONS. Thymus glands were removed from the animals following ether anesthesia and decapitation. The cells were released from the glands by forcing the tissue (4–5 glands together with 5–6 ml of Robinson's medium) through a series of syringes of decreasing size, and eventually through a BD-18 needle. Fibrous tissue was removed by filtration through surgical gauze. In some experiments the resulting suspension was used directly, and is referred to in the text as unwashed cells. To obtain washed cells, the above suspension was centrifuged for 6–7 min at 0°C, 1000 X g. The packed cells were resuspended in fresh medium and filtered through gauze. Aliquots of the suspensions were added to the incubation vessels. The yield of unwashed cells was 240–300 mg/rat, and of washed cells, 175–225 mg.

CHLOROLEUKEMIA. Cell suspensions were prepared from the tumor by the procedure used for thymus. The tumor was used 20–25 days after transplant, at which time the tumor weighed 10–12 gm.

EHRlich ASCITES CELLS. The ascitic fluid was aspirated from the peritoneal cavity of tumor-bearing mice, and centrifuged for 5 min at 0°C, 1000 X g. The packed cells were resuspended in 5–6 volumes of Robinson's medium, and aliquots were added to the incubation vessels. The tumors were used 8–10 days after transplant.

Conditions of Incubation

Sodium formate-14C (Merck & Co., Montreal) was dissolved in distilled water and added to the incubation flasks to provide a final concentration of 3.2 µCi/ml. Aliquots of cell suspensions containing 130–170 mg of cells (wet weight) were incubated with formate in a total volume of 2.5 ml in 50-ml Erlenmeyer flasks. The alkaloids, as the sulfates, were dissolved in Robinson's medium and added to the flasks in amounts to provide the final concentrations of free base noted in the text and tables. Control flasks contained an equal volume of Robinson's medium. The flasks were stoppered with cotton plugs and incubated at 37°C in an Eberbach water-bath shaker, with air as the gas phase, at a shaking rate of 120–130 oscillations per min. Under these conditions the rate of incorporation of sodium formate-14C into the DNA of thymus cells was linear for 6 hr (Chart 1).

At the end of the incubation the flasks were placed in ice and their contents were poured into chilled centrifuge tubes. Each flask was rinsed with cold medium. If analysis was not started immediately, the tubes were stored at −20°C.

Analytical Methods

SEPARATION OF RNA AND DNA. Following removal of the acid-soluble material (in 0.7 N perchloric acid) and extraction of lipids, the nucleic acids were isolated from the tissue by the salt extraction procedure of Hecht and Potter (8). RNA and DNA were separated by alkaline degradation of RNA to the 2'-3' nucleotides, followed by acid precipitation of DNA. The DNA precipitate was dissolved in ammonium hydroxide for chemical analysis and measurement of radioactivity.

CHEMICAL DETERMINATIONS. RNA- and DNA-phosphorus were determined by the method of Allen (1). The ribose in the RNA fraction was determined by the orcinol method (14). DNA was determined by the Ceriotti method (4), with highly polymerized calf thymus DNA (Sigma Chemical Co.) as standard.

ISOLATION OF THE PURINE AND PYRIMIDINE BASES FROM RNA AND DNA. Following chemical and radioactivity measurements, the bases of the RNA and DNA fractions were liberated by perchloric acid hydrolysis. Excess perchloric acid was removed as KClO4, and the bases in the supernatant fraction were separated by paper chromatography in the isopropanol-HCl-H2O system of Wyatt (23). The positions of the bases were determined by examination in ultraviolet light. After elution from paper with 0.1 N HCl, the bases were rechromatographed, a butanol-NH4H system (13) being used for adenine and thymine, and a butanol-acetic acid system (21) for guanine. The bases were again eluted, and their spectra determined, against appropriate paper blanks, in a Cary recording spectrophotometer, Model 11.

CHART 1.—The incorporation of sodium formate-14C into the DNA of thymus cells. The incubation system is described in the text.
TABLE 1
EFFECT OF VINBLASTINE (VLB) ON SODIUM FORMATE-^{14}C INCORPORATION INTO THE NUCLEIC ACIDS OF DIFFERENT TISSUES

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>TYPE OF EXPERIMENT</th>
<th>DOSE OR CONCENTRATION OF VLB</th>
<th>SPECIFIC ACTIVITY (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Regenerating rat liver</td>
<td>In vivo (^b)</td>
<td>1 mg/kg</td>
<td>130</td>
</tr>
<tr>
<td>Ehrlich ascites cells</td>
<td>In vivo (^b)</td>
<td>6 mg/kg</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>In vivo (^b)</td>
<td>100 μg/ml</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>In vivo (^b)</td>
<td>0.6 mg/kg</td>
<td>102</td>
</tr>
<tr>
<td>Rat bone marrow</td>
<td>In vivo (^b)</td>
<td>50 μg/ml</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>In vivo (^b)</td>
<td>10 μg/ml</td>
<td>58</td>
</tr>
<tr>
<td>Rat thymus cells</td>
<td>In vivo (^b)</td>
<td>50 μg/ml</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>In vitro (^b)</td>
<td>100 μg/ml</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>In vitro (^b)</td>
<td>1.5 mg/kg</td>
<td>51</td>
</tr>
<tr>
<td>Rat chloroleukemia</td>
<td>In vivo-in vitro(^b)</td>
<td>0.5 mg/kg</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>In vitro</td>
<td>100 μg/ml</td>
<td>75</td>
</tr>
</tbody>
</table>

\(^a\) Time of incubation in vitro, 3 hr; the system in vitro is described in the text.
\(^b\) Results reported previously (2).
\(^c\) Results reported previously (18). Animals were treated with VLB and thymus cell suspensions prepared 15 hr later. These cells were then incubated for 3 hr under conditions outlined in “Methods,” without further addition of alkaloid.

Concentrations of the bases were calculated by using established extinction coefficients.

RADIOACTIVITY MEASUREMENTS. Aliquots of the RNA and DNA solutions were evaporated on aluminum planchets and counted in a Nuclear-Chicago windowless gas flow counter. At least 5000 counts were accumulated for each sample. Two methods were used for determining the radioactivity of the purine and pyrimidine bases. Aliquots of the HCl eluates were either evaporated on platinum planchets and counted as above, or added to vials for counting in the Packard Tri-Carb liquid scintillation spectrometer. The vials contained 1 ml of hyamine (1 M in methanol), and 9 ml of scintillant fluid, composed of 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazolyl)-benzene in a 60:40 mixture (by volume) of toluene-ethanol. All counts were corrected to values obtained by plate counting. It was possible to account for 85-110% of the counts in the DNA fraction on the basis of the specific activities of the purines and pyrimidines, and published figures (5) for the base composition of rat thymus DNA.

From the chemical and radioactivity measurements, the specific activities of RNA and DNA were calculated as follows: DNA, cpm/μg DNA; DNA, cpm/μg DNA-P; RNA, cpm/μg ribose; and RNA, cpm/μg RNA-P.

The effects of VLB and other agents are expressed as percentage of control, e.g.,

\[
\frac{\text{specific activity of DNA of treated cells}}{\text{specific activity of DNA of control cells}} \times 100
\]

In the tables, the “% of control” represents the average of the effects as determined on DNA and DNA-P. The 2 methods gave results agreeing within 10%.

Results
Effect of VLB on sodium formate-^{14}C incorporation by different tissues

The results in Table 1 show that VLB is selective in its effect on animal tissues in vivo. The incorporation of sodium formate^{14}C into the nucleic acids of regenerating rat liver and Ehrlich ascites in mice was not affected by treatment of the animals with VLB. In contrast, the incorporation of isotope into rat bone marrow cells, and particularly into the DNA, was markedly depressed by administration of the alkaloid.

The same tissue specificity was observed under conditions in vitro. The incorporation of sodium formate-^{14}C by rat bone marrow cells was decreased in the presence of VLB, but the same process was not affected in the Ehrlich ascites cells in vitro, even at a higher concentration of the drug. The chloroleukemia, like the Ehrlich ascites, is considered to be sensitive to VLB (15), but, also like the Ehrlich tumor, treatment must be started before the tumor has become well established in the host. The results in Table 1 show that formate incorporation by this tumor, in vitro, was also relatively insensitive to VLB.

Although rat bone marrow is sensitive to VLB in vivo and in vitro, it is not a convenient tissue for biochemical studies. For this reason the effect of VLB on sodium formate-^{14}C incorporation by rat thymus cells in vitro was determined. The results in Table 1 show that this process is sensitive to VLB in these cells. At all concentrations tested, incorporation of isotope into nucleic acids was decreased in cells incubated with VLB. The qualitative and quantitative effects of the alkaloid on thymus cells in vitro were very similar to the effect on bone marrow cells under the same conditions. The decreases in labeling of RNA and DNA were the same in both tissues, and, in each cell type, the incor-
poration of isotope into DNA was inhibited more than the incorporation into RNA. This difference was observed at all concentrations of VLB. A direct comparison of the effect of VLB on isotopic incorporation into nucleic acids by thymus cells in vivo and in vitro has not been obtained. However, preliminary experiments show that thymus cells from animals treated with nontoxic amounts of VLB have a greatly impaired ability to incorporate sodium formate-14C into nucleic acids under conditions in vitro (18) (Table 1). Since rat thymus cells are more readily obtained in adequate amounts and respond to VLB in the same way as bone marrow cells, subsequent experiments were performed with that tissue.

**Effect of Other Alkaloids on Sodium Formate-14C Incorporation by Thymus Cells in vitro**

Since alkaloids, as a class, can cause protein precipitation, it was advisable to see if the inhibitory action of VLB was an example of this general phenomenon or was due to a more specific effect of the compound. The effects of the 4 Vinca alkaloids of chemotherapeutic interest, and of reserpine, another indole compound, on the thymus system were determined. The results in Table 2 show that, at the concentration used, reserpine and vinrosidine did not affect the labeling of the nucleic acids, whereas a substantial inhibition was noted with vinueurosine, VLB, and vincristine. In each case in which inhibition was observed, labeling of DNA was affected more than that of RNA. These results indicate that the inhibition produced by these compounds was not due to a general chemical property of alkaloids, but may be a specific function of these particular compounds.

**Effects of VLB on Sodium Formate-14C Incorporation by Washed and Unwashed Thymus Cells**

For biochemical studies, it is customary to wash the cells obtained from tissues to remove debris and material from cells which were disrupted during the preparative procedure. However, in studying the effects of a drug on cell suspensions, the removal of an essential cofactor during the washing procedure could alter the nature and magnitude of the effect. To investigate this possibility, the effects of VLB on sodium formate-14C incorporation into the nucleic acids of washed and unwashed cell suspensions were compared over periods of 0.5-3 hr.

The results in Chart 2 show that the incorporation of sodium formate-14C into DNA was very similar in washed and unwashed cell preparations, and the final specific activities in control cells were almost identical. The effect of VLB on the labeling of DNA was also similar in the 2 types of cells. Some inhibition was observed at 30 min, and at 3 hr there was a 55-60% inhibition of the incorporation of sodium formate-14C into the DNA of washed and unwashed cells incubated with the alkaid. The effects of VLB on the incorporation of isotope into RNA were also similar in the 2 cell preparations, both showing 35-40% inhibition at 3 hr. However, the specific activities of the RNA from the 2 types of cells were markedly different. Although the counts incorporated per mg of tissue were the same, the specific activity of the RNA from washed cells was much higher than that of RNA from unwashed cells. On analysis, it was found that washed cells contain much less RNA than unwashed cells. In a series of experiments it was observed that the washed cells contained only 55-70% of the RNA of unwashed cells, based on the RNA-ribose and RNA-P content per mg of tissue. There was no difference in DNA content. Similar findings have been reported by Roof and Aub (20). The results of several experiments showing the effect of washing on DNA and RNA content and the DNA-P/RNA-P ratio of thymus cells are shown in Table 3. The difference in RNA content of washed and unwashed cells offers an explanation for the difference in the specific activity of RNA in the 2 preparations. The presence of a larger amount of RNA in unwashed cells would dilute the radioactivity incorporated by the tissue.

### Table 2

<table>
<thead>
<tr>
<th>ALKALOID</th>
<th>SPECIFIC ACTIVITY (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA</td>
</tr>
<tr>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>60</td>
</tr>
<tr>
<td>Vinueurosine</td>
<td>63</td>
</tr>
<tr>
<td>Vincristine</td>
<td>84</td>
</tr>
<tr>
<td>Vinrosidine</td>
<td>92</td>
</tr>
<tr>
<td>Reserpine</td>
<td>103</td>
</tr>
</tbody>
</table>

* The incubation system is described in the text. Concentration of alkaloids, 50 μg/ml; time of incubation, 3 hr.
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which was the same in both cell types. Thus the specific activity would be lower in the unwashed cells.

In considering the action of VLB, it is important to note that the effect of the alkaloid on the incorporation of sodium formate-\(^{14}\)C into the nucleic acids is the same in washed and unwashed cells. Supporting evidence is shown in Table 4. These results are the averages of several experiments, and show that the effects of VLB on labeling DNA, RNA, and their constituent purine and pyrimidine bases are the same in the 2 cell types. Also significant is the similarity of the specific activities of the various components, other than RNA and the RNA-purines, in the 2 systems. Washed cells have been chosen for subsequent work, since this type of cell suspension is more uniform, microscopically, from experiment to experiment. Unwashed preparations show varying degrees of cell clumping, as well as different amounts of cell debris. Since isotope incorporation was equally affected, the washed cells have been chosen because of the ease in obtaining a standard preparation.

**Effect of VLB on Labeling of DNA Bases at Different Time Intervals**

The results in Table 4 also show that after 3-hr incubation, the specific activity of the DNA-thymine of cells incubated with VLB was decreased, relative to that from control cells, to about 50%. However, at that time, the specific activity of the DNA-purines from treated cells were decreased to a somewhat greater extent, to about 35% of controls. It seemed advisable to determine whether this differential response to VLB was established at once or developed gradually during the incubation. The results in Table 5 show the effect of VLB on the labeling of the total DNA fraction and of DNA-adenine, -guanine, and -thymine after different periods of incubation. The figures show that there was a significant decrease in the specific activities of the DNA-purines before there was a marked inhibition of labeling of DNA-thymine. These results suggest the possibility that VLB may affect specifically the synthesis or incorporation of DNA-purines.

**Discussion**

The experimental results show that in 2 biologically sensitive tissues, bone marrow and thymus, the incorporation of sodium formate-\(^{14}\)C into RNA and DNA is decreased by VLB under conditions in vitro. The amounts of VLB used in these studies are relatively high. However, the qualitative and quantitative similarity of the response in vitro to that obtained with bone marrow in vivo and with thymus cells from animals treated with nontoxic amounts of VLB (18) suggests that the mechanism of action of the alkaloid is the same under the different types of experimental conditions. This requirement for a larger amount of VLB in vivo may be linked to the metabolism of the drug. It is possible that VLB is changed to a biologically more active compound in the intact animal, and the extent of this alteration may be less in thymus cells in vivo.

Similar reasoning may explain the quantitative differences between the biologic effects of the various Vinca alkaloids in vivo and their effects on nucleic acid formation in thymus cells in vitro. For example, vinleurosine is required in much larger amounts than VLB to produce a therapeutic response in animal tumors, yet the 2 compounds were equally effective with the thymus system. If vinleurosine were degraded or excreted more rapidly than VLB in intact animals, larger doses would be required to elicit a therapeutic response. This need not apply to experiments in vitro, wherein the alkaloids were added directly to the tissue preparation. Other studies support this hypothesis. The ability of thymus cells from vinleurosine-treated rats to

**TABLE 3**

**Effect of Washing Cells on the Nucleic Acid Content of Rat Thymus Cells**

<table>
<thead>
<tr>
<th>Type of cell preparation</th>
<th>DNA content (% of wet weight)</th>
<th>RNA-ribose content (% of wet weight)</th>
<th>Ratio, DNA-P/RNA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unwashed cells</td>
<td>2.91 ± 0.15</td>
<td>0.12 ± 0.01*</td>
<td>4.3/1*</td>
</tr>
<tr>
<td>Washed cells</td>
<td>2.96 ± 0.17</td>
<td>0.07 ± 0.01*</td>
<td>7.1/1*</td>
</tr>
</tbody>
</table>

* The \( P \) value for differences between washed and unwashed cells was <0.01.

**TABLE 4**

**Effect of Vinblastine (VLB) on Sodium Formate-\(^{14}\)C Incorporation into Nucleic Acids of Washed and Unwashed Thymus Cells In Vitro**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Measurement</th>
<th>UNWASHED CELLS</th>
<th>WASHED CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Effect of VLB (% of control)</td>
<td>Control</td>
</tr>
<tr>
<td>DNA</td>
<td>cpm/μg DNA</td>
<td>24.4</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>cpm/μg DNA-P</td>
<td>325.3</td>
<td>46.4</td>
</tr>
<tr>
<td>DNA-thymine</td>
<td>cpm/μg ribose</td>
<td>102.1</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>cpm/μg RNA-P</td>
<td>239.6</td>
<td>67.9</td>
</tr>
<tr>
<td>DNA-adenine</td>
<td>cpm/μmole</td>
<td>25,130</td>
<td>52.6</td>
</tr>
<tr>
<td>DNA-guanine</td>
<td>cpm/μmole</td>
<td>5,450</td>
<td>36.1</td>
</tr>
<tr>
<td>RNA-guanine</td>
<td>cpm/μmole</td>
<td>6,185</td>
<td>34.8</td>
</tr>
<tr>
<td>DNA-adenine</td>
<td>cpm/μmole</td>
<td>11,465</td>
<td>72.2</td>
</tr>
<tr>
<td>DNA-guanine</td>
<td>cpm/μmole</td>
<td>6,715</td>
<td>70.4</td>
</tr>
</tbody>
</table>

* The incubation system is described in the text. Concentration of VLB was 50 \( μ \)g/ml. Results are averages of several (10-15) experiments of 3-hr duration.
incorporate sodium formate-\(^{14}\)C into nucleic acids in vitro is not impaired nearly as much as that of cells from animals given an equal amount of VLB (18).

It is difficult to explain the tissue specificity of sensitivity to VLB. The biosynthetic pathways for RNA and DNA formation are apparently identical in different tissues, yet marked differences in the effects of VLB on the incorporation of sodium formate-\(^{14}\)C into nucleic acids have been observed (Table 1). It is possible that the effect of VLB on nucleic acid formation is not the primary site of drug activity. Rather, the alkaloid may act on some other metabolic process which then influences nucleic acid formation. A detailed investigation of the effect of VLB on nucleic acid formation might reveal such a relationship. Initial studies on the effect of VLB on the synthesis of nucleic acids and their precursors are reported in a separate communication.

Acknowledgments

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