Biochemical Aspects of Leukemia Virus-induced Immunosuppression: Effect of Friend Disease Virus on Spleen Nucleases and Nucleic Acids

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SUMMARY

The effect of Friend disease virus infection on spleen acid nuclease activity was studied in detail. RNase activity decreased rapidly, with time, during the course of the virus-induced leukemogenesis. Within 3 to 12 days after infection there was an 80 to 90% or greater suppression of RNase activity, as calculated per mg spleen tissue or protein. RNase activity remained depressed throughout the net period of 12 days. In contrast, DNase activity decreased sharply during the first 3 days after infection and then returned to near normal levels.

There was little or no change of total DNA content per mg protein spleen tissue in infected mice. However, RNA levels decreased moderately and then increased sharply during the 1st week of infection.

Studies on the subcellular distribution of nucleases and nucleic acids indicated that there was little, if any, detectable RNase in the nuclear, mitochondrial, and microsomal fraction of spleens of infected mice, as compared to the significant amounts of this enzyme in similar fractions of normal spleens. Most of the RNase activity in infected spleens was found only in the supernatant fraction.

There was little significant difference in the localization, distribution, and quantity of DNase activity in subcellular splenic fractions of normal or control animals. The only difference was the slight decrease in DNase activity in the nuclear and supernatant fractions in infected spleens.

Most of the RNA in the spleens of both infected and control animals was in the microsomal fractions. However, there was twice as much RNA in this fraction with infected spleens, as compared to controls. There was little or no significant difference in the DNA content of the various subcellular fractions of normal or infected animals.

INTRODUCTION

Friend disease leukemia in mice has been studied extensively as a model for virus-induced leukemogenesis (15, 22, 25). In recent years it has been reported from a number of laboratories, including our own, that leukemogenic viruses, such as FDV, may induce a marked immunological deficiency in infected animals (3–6, 9, 16, 24, 27–29). For example, previous studies from our laboratory have shown that mice given injections of FDV virus 1 or 2 days prior to challenge immunization with an antigen such as sheep erythrocytes or Escherichia coli lipopolysaccharide have a 95% or greater depression of the expected immune response, as detected on both the cellular and humoral antibody level (3–6, 16). Such suppression generally occurs prior to development of overt splenomegaly. When virus is injected 1 week or longer before immunization, there is a marked increase in spleen size as well as an even greater depression of the immune response.

The mechanism whereby a leukemogenic virus such as FDV results in immunological deficiency is not known. However, it seems probable that the virus may have as its target the same stem cells which are involved in immunogenesis (5, 6, 29). Little is known about the exact nature of such precursor cells, especially the biochemical pathways involved in their stimulation and differentiation into antibody-producing cells. In this regard, many studies in recent years have shown that RNA metabolism is extremely important during the synthesis of antibody, as well as other proteins. There is a significant increase in the quantity of nucleic acids in lymphoid tissues of immunized individuals. In addition, it is thought that a specific form of RNA, possibly associated with a persisting antigenic determinant or in the form of a specific antigen-RNA complex, may be involved in immunity (14).

The role of nucleic acid metabolism is also important in oncogenesis. There is a marked increase in nucleic acid content of spleens of mice infected with a leukemia virus such as FDV (12, 23). Probably a similar mechanism is involved in the enhancement of nucleic acids in spleens of mice infected with a leukemia virus and those stimulated with an antigen. In a preliminary study, it

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2 The abbreviations used are: FDV, Friend disease virus; TCA, trichloracetic acid; RBC, red blood cells; PFC, plaque-forming cells.

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was observed that the level of nucleic acids, as well as nuclelease activity, changed rapidly during antibody formation (8). A possible role for nucleases as a controlling factor for RNA metabolism was suggested. A similar relationship between nuclelease activity and RNA metabolism in leukemic animals may be important.

Various studies have been concerned with biochemical aspects of lymphoid tissues in mice infected with leukemia viruses such as FDV. Increased protein synthesis, as well as nucleic acid synthesis, occurs rapidly in spleens of infected mice (2, 30). In a recent report, mainly concerned with nucleic acid metabolism, a marked decrease in RNase activity was observed in spleens of FDV-infected mice (30). The present report presents results describing in greater detail the changes in RNase and was also studied.

The effect of infection on total RNA and DNA content of the spleen and subcellular fractions was also studied.

**MATERIALS AND METHODS**

**Experimental Animals**

Female BALB/c mice, generally 5 or 6 weeks old at the start of an experiment, were obtained from Flow Laboratory Animals, Dublin, Va. They were housed in groups of 6 in plastic cages and fed mouse pellets and water *ad libitum*.

**Infection**

Mice were infected by i.p. injection of FDV. The virus was obtained from the American Type Culture Collection and passaged in BALB/c mice, as described elsewhere (3-6). Experimental animals were infected i.p. with 0.5 ml clarified 10% cell-free extract prepared from the stock virus preparation. The mice were observed for development of leukemia at periodic intervals. Splenomegaly was used as the major indicator of leukemia (4-6). However, selected spleen sections were examined histologically throughout the course of the study.

**Preparation of Spleen Homogenates**

Five or more infected mice, as well as an equal number of control mice, were sacrificed at various time intervals after infection, such as 1, 3, 6, and 9 days. The spleens were quickly excised and weighed.

For immunological studies, described below, other spleens were left unfrozen. For enzyme assay spleens pooled from each group were frozen with Dry Ice and stored at -60° until used, when they were homogenized at 4° with 3 to 5 volumes 0.1 M KCl for 5 min in a Tri-R homogenizer at maximum speed. An aliquot of each extract was used for enzyme determination, as described below. Freezing had no effect on enzyme activity.

**Fractionation of Spleen and Preparation of Ribosomes**

Spleen homogenates were treated so as to prepare 4 subcellular fractions, essentially according to the procedure of Tashiro and Siekevitz (31). A 10% (w/v) spleen cell homogenate was prepared with cold sterile 0.25 M sucrose, pH 7.6, containing 0.005 M Cl2, 0.05 M KCl, and 0.025 M Tris buffer. The nuclear fraction was separated by low-speed centrifugation at 800 × g for 15 min in the cold. The nuclei-containing pellet was washed twice with cold buffer and the washings were added to the supernatants. The mitochondria-rich fraction was obtained from the supernatant by centrifugation at 10,000 × g for 15 min in the cold. The resulting supernatant was then centrifuged at 105,000 × g in the cold for 2 hr in a Spinco Model L centrifuge to obtain the microsome-rich fraction. Each of these fractions was restored to a volume of 1 to 2 ml with cold 0.1 M KCl. There was approximately a 90 to 95% recovery of total protein of the original extracts.

The ribosome fraction was prepared by suspending the microsome pellet in 0.05 M Tris buffer, pH 7.6, containing 0.001 M MgCl2, followed by homogenization in the cold for 1 min. Sodium deoxycholate was added to the homogenate to a final concentration of 0.5%, followed by centrifugation at 105,000 × g at 4° to precipitate the ribosomes. The resulting pellet was suspended in 0.001 M Tris buffer, pH 7.6, containing 0.001 M MgCl2. The preparation was then centrifuged at 10,000 × g for 15 min in the cold to remove large aggregates. The supernatant was considered to contain the ribosomes.

**Enzyme Assays**

**Ribonuclease.** RNase was assayed, as follows, with the use of MacFayden reagent and the method of Eichel, with several modifications (13). The substrate consisted of 2 mg yeast RNA (Calbiochem, Los Angeles, Calif.) per ml 0.07 M Veronal-acetate buffer, pH 5.8, containing 0.15 M KCl. A standard aliquot of the test material was added to this reaction mixture and incubated for 30 min at 38°. The reaction was stopped by addition of 0.75% uranyl acetate in 0.25% perchloric acid solution. The reaction tubes were then centrifuged in the cold for 30 min at 3000 × g. An aliquot of the clear supernatant was diluted with a standard quantity of distilled water and examined spectrophotometrically at 260 μm. The results of the reagent blank and the tissue extract blank were subtracted from the results obtained with the test samples. A unit of RNase activity was defined as an increase of 0.01 unit absorbance at 260 μm. Specific activity of the enzyme was defined as 1 unit/mg protein. Enzyme activity was determined in the linearity range.
Deoxyribonuclease. DNase activity in spleen extracts and fractions was assayed with 0.12 M acetate buffer, pH 5.0, in the presence of Mg"+, by several modifications of the method of De Duve et al. (11). The substance was polymerized sperm DNA. After incubation of the reaction mixture for 30 min at 37°, 0.5 ml cold 2 N HCl was added to each tube. After 15 min, the tubes were centrifuged for 30 min at 3000 × g at 4°. The absorbance of an aliquot of the clear supernatant, diluted with sufficient distilled water, was determined at 260 m$. The results of the reagent blank and tissue blank were subtracted from the results of the test samples and the difference was recorded. A unit of DNase was defined as an increase of 0.01 unit absorbance at 260 m$. Specific activity of the enzyme was recorded as units/mg protein.

Protein, DNA, and RNA Determinations

Protein was determined by the method of Lowry et al., with bovine serum albumin as the standard (20). Ribonucleic acid was determined by the orcinol method, with yeast RNA as the standard (21). DNA was determined by the indole method, with sperm DNA as the standard (7). The presence of sucrose used to prepare subcellular fractions interfered with nucleic acid determinations. Therefore, sucrose was removed from the fractions by exhaustive washing with distilled water. Protein in the subcellular fractions was precipitated with cold 5% TCA. The pellet was obtained by centrifugation, homogenized with water, and reprecipitated with 5% TCA. Three consecutive washings removed all detectable traces of sucrose. No detectable protein or nucleic acid was removed.

Immunology

For immunological studies, 5 or more mice were infected at various time intervals prior to i.p. injection of 0.5 ml 10% suspension of freshly washed sheep RBC. The mice were sacrificed 4 days later, at the time of the expected peak immune response, and the spleens were obtained and "teased" with needles and forceps to prepare a dispersed cell suspension, as described previously (3-6). The number of viable nucleated cells was determined for each suspension using a hemocytometer and trypan blue supravital stain technique. An aliquot of each cell suspension was tested for the number of specific antibody PFC to the sheep RBC's by the localized hemolytic plaque procedure in agar gel, as described previously (3-6). In brief, the cells were incubated at 37° in agar plates containing target erythrocytes and complement. Development of localized zones of hemolysis was the indicator of the presence of hemolysin-forming cells. The number of such cells was determined by counting the plaques on 3 or more plates, with several cell concentrations. The number of PFC's per million leukocytes plated and per whole spleen was determined for each animal.

Experimental Design

Groups of mice were given injections of FDV at various times before sacrifice or immunization. Equal numbers of infected and control mice were then sacrificed at a given time; the spleens were removed, weighed, and used for determination of splenic RNase, DNase, protein, DNA, and RNA. The number of PFC's per spleen and per million splenic leukocytes was determined with the groups of mice specifically immunized at various times before sacrifice. The level of nuclease activity was correlated with the day of infection, the degree of splenomegaly, and the immune response of the animals. In addition, subcellular distribution of nuclease activity, protein, DNA, and RNA was determined for each experimental group. For this purpose, the spleen cell homogenates were subjected to differential centrifugation to obtain the nuclear, mitochondrial, microsomal, and cell supernatant fractions. The ribosome-rich fraction was isolated from the microsomes and similarly tested for nuclease activity.

RESULTS

The Relationship between Splenomegaly and Immunosuppression. As can be seen from Chart 1, there was a rapid development of splenomegaly after infection of the mice with FDV. Normal control mice had a spleen weight of approximately 150 mg. No control animal had
A. K. Chakrabarty, H. Friedman, and W. S. Ceglowski

a spleen weighing more than 200 mg. There was little change during the first 2 days after infection. There was a moderate increase in spleen weight by Days 3 to 4, with a continued rise thereafter, so that by Day 12 the average spleen weight was about 1400 mg, with a range from 1100 to 1700 mg. The total amount of protein per whole spleen increased several-fold during the first 1 or 2 weeks after infection (Table 1). However, there was no relative or absolute change of protein per mg tissue during this time. There was almost a direct inverse relationship between the time of infection and the immune response of the mice to sheep erythrocytes. As observed previously, infection of mice the day of immunization had little, if any, suppressive effect on the number of PFC's detected 4 days later. However, when mice were infected 1 day prior to immunization, there was moderate decrease in the number of PFC's. When there was a 3-day interval between infection and immunization there was a greater than 60% suppression. Mice infected 1 to 2 weeks prior to immunization had a 90% or greater suppression in the number of splenic PFC's, as compared to control animals.

**RNase Activity in Spleens of Infected Mice.** RNase activity decreased rapidly, with time, during the course of FDV-induced leukemogenesis (Table 2, Chart 2). In a typical experiment there was a 10 to 20% reduction in the level of RNase, as determined either per mg protein or per 100 mg spleen weight, during the first 3 days after infection (Table 2). By Day 6 there was an 80 to 90% decrease. The lowest activity occurred in spleens of mice infected for 9 to 12 days (Table 2). When enzyme activity was calculated as activity per whole spleen, there was approximately a 35 to 45% suppression of activity as compared to normal spleens in 9- to 12-day infected mice (Table 2).

**RNA Content in Spleens of Infected Mice.** There was a moderate decrease in the amount of RNA per mg spleen protein or per 100 mg spleen in mice infected 3 days previously with FDV (Table 3, Chart 2). However, by Days 4 to 5 the level of RNA returned to normal, and then increased rapidly so that by Days 6 to 12 there was a 40 to 50% enhancement of RNA in spleens of infected mice, as compared to spleens of controls. The total level of RNA per intact spleen paralleled the rise in spleen size and increased 6- to 8-fold during the first 12 days of infection.

**Effect of FDV Infection on Splenic DNase Activity and DNA.** As can be seen from Table 4, there was a marked decrease in the level of DNase activity in spleens of mice during the first few days after infection. How-

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**Table 1**

*Effect of FDV infection on spleen protein*

<table>
<thead>
<tr>
<th>Mouse group*</th>
<th>Spleen protein (mg)</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25.0</td>
<td>100</td>
<td>12.0</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+3 day</td>
<td>28.2</td>
<td>113</td>
<td>12.1</td>
</tr>
<tr>
<td>+6 day</td>
<td>100.0</td>
<td>400</td>
<td>12.2</td>
</tr>
<tr>
<td>+9 day</td>
<td>150.0</td>
<td>600</td>
<td>11.2</td>
</tr>
<tr>
<td>+12 day</td>
<td>160.0</td>
<td>640</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Groups of 5 mice each were sacrificed on the day indicated after infection with FDV and protein content per whole spleen or per 100 mg spleen weight was determined.

**Table 2**

*Effect of FDV infection on total splenic RNase, as well as units/100 mg tissue or per mg protein*

<table>
<thead>
<tr>
<th>Mouse group*</th>
<th>RNase (pH 5.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3625 100 1980 100 145 100</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
</tr>
<tr>
<td>+3 day</td>
<td>3708 102 1640 83 131 90</td>
</tr>
<tr>
<td>+6 day</td>
<td>2000 56 240 12 20 13</td>
</tr>
<tr>
<td>+9 day</td>
<td>2375 65 170 8 16 11</td>
</tr>
<tr>
<td>+12 day</td>
<td>2255 62 181 9 14 9</td>
</tr>
</tbody>
</table>

* Groups of 5 or more mice were sacrificed on the day indicated after infection with FDV, and spleens were pooled for enzyme assay.

One unit, 0.01 absorbance change.

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![Chart 2. Effect of FDV infection on splenic RNase, DNase, RNA, and DNA. Each point represents average results of 5 or more mice, tested on the indicated day, as percentage of control values for noninfected animals. The nuclease and nucleic acid activities were calculated per mg spleen.](chart2.png)
Virus-induced Immunosuppression

ever, DNase activity rapidly returned to a normal level so that by Day 12 after infection there was generally less than a 20 to 30% difference between spleens of infected and control mice (Chart 2). The relative content of DNA per mg protein or per 100 mg spleen homogenate of infected mice did not differ markedly from that of the controls (Table 5, Chart 2). However, DNA content did increase per whole spleen during the course of infection. This increase was not as rapid as that of RNA and protein.

**Subcellular Distribution of Nuclease Activity.** Since the RNase level was markedly low in spleens of infected animals, both when calculated per 100 mg tissue or per mg protein, as well as per whole spleen, it was of interest to determine the distribution of enzyme activity in various subcellular fractions. As can be seen from Chart 3, there was little, if any, detectable RNase in the nuclear, mitochondrial, or microsomal fractions of spleens of mice infected 6 to 9 days previously. The supernatant fractions contained nearly all the activity, but this was about half as much as that of the control spleens. Most of the RNase activity in spleens of control mice was found in the mitochondrial and cell supernatant fractions, and about equal amounts in the nuclear and microsome-rich fractions. No RNase activity was detected in the ribosome fractions of spleens of infected mice.

There was little significant difference in the subcellular localization of DNase activity in spleens of infected and control animals. As can be seen from Chart 3, the mitochondrial and microsome-rich fractions of either control or 6- to 9-day infected mice had essentially the same DNase activity. The only difference was the presence of slightly less DNase in the nuclear and supernatant fractions in spleens from infected mice (Chart 3).

### Table 3
**Effect of FDV infection on total spleen RNA, as well as per 100 mg tissue or per mg protein**

<table>
<thead>
<tr>
<th>Mouse group*</th>
<th>RNA (mg)</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2,000</td>
<td>100</td>
<td>966</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Infected</td>
<td>1,667</td>
<td>83</td>
<td>848</td>
<td>87</td>
<td>61</td>
</tr>
<tr>
<td>+3 day</td>
<td>13,333</td>
<td>666</td>
<td>1,624</td>
<td>168</td>
<td>133</td>
</tr>
<tr>
<td>+6 day</td>
<td>16,667</td>
<td>833</td>
<td>1,440</td>
<td>149</td>
<td>141</td>
</tr>
<tr>
<td>+9 day</td>
<td>17,055</td>
<td>852</td>
<td>1,560</td>
<td>161</td>
<td>140</td>
</tr>
</tbody>
</table>

*Groups of 5 or more mice were sacrificed on the day indicated after FDV infection.

### Table 4
**Effect of FDV infection on total spleen DNase activity, as well as units/mg spleen tissue or per mg protein**

<table>
<thead>
<tr>
<th>Mouse group*</th>
<th>DNase (pH 5.0)*</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>875</td>
<td>100</td>
<td>1,290</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>Infected</td>
<td>750</td>
<td>85</td>
<td>322</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>+3 day</td>
<td>6,250</td>
<td>714</td>
<td>760</td>
<td>58</td>
<td>63</td>
</tr>
<tr>
<td>+6 day</td>
<td>11,500</td>
<td>1,314</td>
<td>860</td>
<td>66</td>
<td>76</td>
</tr>
<tr>
<td>+9 day</td>
<td>14,670</td>
<td>1,676</td>
<td>910</td>
<td>70</td>
<td>82</td>
</tr>
</tbody>
</table>

*Groups of 5 or more mice were sacrificed on the day indicated after FDV infection and spleens were pooled for enzyme assay.

*One unit, 0.01 absorbance change.
Subcellular Distribution of RNA and DNA. Most of RNA in spleens of both infected and control animals was in the microsome-rich fraction (Chart 4). However, there was twice as much RNA in this fraction prepared from spleens of infected mice, as compared to controls. There were similar quantities of RNA in the nuclear, mitochondrial, and supernatant fractions of spleens of infected and control animals. Likewise, there was little or no significant difference in the DNA content in the nuclear fractions of control and infected animals (Chart 4). There was a moderate but similar quantity of DNA in the mitochondrial fractions of both groups. Little, if any, DNA was found in other fractions.

DISCUSSION

The results of the present study indicate that significant biochemical changes occur in the spleen of mice infected with a leukemogenic virus such as FDV. After a latent period of 2 to 4 days, the spleen weight of infected mice increases rapidly so that within 12 days there is an 8- to 10-fold enlargement of splenic size. Development of overt splenomegaly is related to a markedly decreased ability of the infected animals to synthesize specific antibody. Such infection also alters basic nucleic acid metabolism patterns. As the results of this study indicate, acid RNase activity decreases rapidly during the first few days after infection, preceding overt splenomegaly but concomitant with development of the immunological deficiency. The maximum decrease of RNase activity occurred between the 3rd and 6th day after infection. By the 12th day RNase activity in infected spleens was about 10 to 15% of the control level and did not change appreciably thereafter.

Other investigators have similarly observed that RNase activity may be diminished in rapidly growing tumors (10, 18, 26). Silber et al. (30) reported a rapid decrease in acid RNase activity during the 1st week after infection of mice with FDV. Ambellan and Hollander (1) have shown that RNase activity is restored during tumor regression. Such results suggest that rapid proliferation of neoplastic cells may be causally related to decreased nuclease activity.

It is also generally agreed that nucleic acid metabolism of a target tissue may be markedly altered by a tumorigenic virus. Silber et al. (30) reported that RNA synthesis increases rapidly in spleens of mice following infection with a tumor virus, returning to normal levels about the 6th day. In the present study, it was observed that the total RNA content of a mouse spleen, after an initial decrease, increased rapidly during the 1st week after infection, reaching a level plateau during the subsequent week. It seems probable that during the later phases of leukemogenesis RNA molecules may be metabolized at a slower rate than in normal animals. Preservation of RNA may be necessary for maintaining the high rate of protein synthesis, associated with rapid cell division.

Results of the present study also indicate that total DNA remained relatively constant during the time when RNA and RNase activity changed rapidly. On the other hand, acid DNase activity decreased during the first 3 days of infection and then gradually returned to normal levels within the next 10 days. The significance of such changes is unknown. However, there have been reports of a marked increase in DNA content in rapidly growing hepatomas but no change in the slowly growing ones (17). It was also reported that DNA levels were not altered in regenerating liver, which has a high rate of cellular proliferation (17). In leukemic mice, where erythroblastosis causes massive splenomegaly, no change in splenic DNA level may indicate an absence of polyploidy in the tumor cells. Furthermore, since FDV induced little or no change in relative DNA content of infected spleens, it appears that a balanced control mechanism exists for DNA metabolism during viral leukemogenesis, in contrast to RNA metabolism or synthesis.

The importance of the more pronounced and prolonged decrease in RNase activity, calculated either per whole spleen or per mg protein, as compared to DNase activity changes, is unknown. However, it seems probable that an increase in RNase activity, rather than a decrease, might interfere with formation of new RNA molecules, which may be necessary for adequate metabolic and cellular changes induced by the virus. In addition to the rapid proliferation of leukemic leukocytes, as well as erythroid cells, in the spleen of infected mice, there is also a rapid increase in the number of viral particles. Thus, it is difficult to determine with any certainty whether biochemical changes associated with tumorigen-
thesis are due exclusively to changes in neoplastic cells only or to actual virus synthesis per se.

The distribution of enzyme activity in several subcellular fractions of the spleen was studied in detail. A marked change in distribution of RNase, but not DNase activity, in the various subcellular fractions could be correlated with infection. Since maximum depression of RNase activity occurred by 9 days after infection, this time was chosen to study the subcellular distribution of the enzyme. A marked decrease in RNase activity was found in all fractions, especially the nuclear and mitochondrial fractions and the cell sap or supernatant fraction. There was no significant difference in the subcellular distribution of DNase or DNA in spleens of control and infected mice. Nearly all measurable DNA was free of detectable RNase activity. There was little or no change in the concentration of total RNA in other fractions, as compared to controls. The increased RNA concentration in the spleen of infected mice was associated mainly with the microsomal fraction. The ribosomes were essentially free of detectable RNase activity. There was little or no change in the concentration of total RNA in other fractions, as compared to control animals.

Lin and Rich (19) have reported an increase in RNA polymerase activity in nuclear fractions of spleens of leukemic mice. There was about 4 times more activity in this fraction than in the cytoplasm. It seems probable that RNA molecules synthesized at a rapid rate in the nucleus may be transferred to microsome fractions for direct involvement in protein synthesis. If this is so, it suggests that rapid lymphoid cell differentiation and proliferation, as well as production of virus particles, markedly influence metabolic pathways of nucleic acid synthesis and utilization. Such changes may be important contributing factors to induction of immunosuppression associated with the leukemic process. Results of other studies to be reported concerning other enzymes involved in amino acid synthesis in spleens of leukemia virus infected mice support such a conclusion.

ACKNOWLEDGMENTS

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A. K. Chakrabarty, H. Friedman, and W. S. Ceglowski

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