Incorporation of Radioactivity from (5-Amino-4-carboxamido-2-¹⁴C-imidazole) adenine Dinucleoside Pyrophosphate into Nucleic Acids*  

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It was previously demonstrated that in the presence of beef spleen nicotinamide adenine dinucleotide glyceraldehyde, 4(5)-amino-4-carboxamidoimidazole reacted irreversibly with nicotinamide adenine dinucleotide to form the pyrophosphate of adenosine 5'-phosphate and of 5(4)-amino-4-carboxamidoimidazole ribonucleotide (i.e. the (5-amino-4-carboxamidoimidazole)-adenine dinucleoside pyrophosphate (1)) (Fig. 1). This dinucleoside pyrophosphate was later converted to hypoxanthine adenine dinucleoside pyrophosphate by semipurified liver extracts (2).

A description of these experiments is reported in this paper.

EXPERIMENTAL PROCEDURE

Materials and Methods—Nucleotides and nucleosides were purchased from Pabst Laboratories and adenine from the following sources: 5'-AMP, 5'-CMP, 5'-GMP, 5'-IMP, and 5'-XMP, all paired with 5'-AMP, were recently prepared in our laboratory by the general method of Khorana (3) and are now available in substrate quantities (R. Reid, A. Papaphilis, and S. G. A. Alivisatos, in preparation for publication). Nutritional Biochemicals Corporation. Pyruvate kinase (ATP: pyruvate phosphorotransferase), 2-phosphoenolpyruvate, and tetrahydrofolic acid were products of the California Corporation for Biochemical Research. Samples of actinomycin D were gifts of Merck Sharp and Dohme Research Laboratories and of Farben Fabriken Bayer A.G. (Germany). Aminopterin and crystalline bovine serum albumin were obtained from Sigma Chemical Company. RNase from bovine pancreas and DNase were products of Worthington Biochemical Corporation. 8-¹⁴C-Labeled nucleotides of adenine and of hypoxanthine were purchased from Schwarz Laboratories, Inc. 5(4)-Amino-4(5)-carboxamido-2-¹⁴C-imidazole was a product of California Corporation for Biochemical Research and was used to prepare (5-amino-4-carboxamido-2-¹⁴C-imidazole)adenine dinucleoside pyrophosphate as described previously (1).

Ehrlich ascites tumor cells, kindly supplied by Dr. R. Baserga, were maintained by serial transplantations in male Swiss mice (6 to 10 weeks old). The cells were harvested 6 to 10 days after inoculation. They were collected in prechilled heparinized tubes, and washed three times by centrifugation (approximately 100 × g) with a solution containing 0.25 μm sucrose and 0.1 μm phosphate buffer, pH 8.1. The washed cells were suspended in the same medium to a volume equal to that of the original ascitic fluid, and homogenized in the cold in a Potter-Elvehjem type homogenizer equipped with a Teflon pestle (Arthur H. Thomas Company). The periods (2 to 3 min) required to achieve rupture of the cells without extensive destruction of the nuclei (4) were checked in a phase microscope. Nuclei were centrifuged down at 600 × g (5), resuspended to the original volume, and further homogenized for 3 min. The 600 × g supernatant was centrifuged in a Spinco model L preparative ultracentrifuge at an average force of 105,000 × g for 1 hour. The clear supernatant and the pellet, brought in suspension in the original volume, were used separately. When the whole homogenate was used for incubation, care was taken to disrupt not only the cells, but also the nuclei. In such instances, disruption was facilitated by suspending the washed cells in 0.05 M Tris-HCl, pH 7.9, prior to homogenization. When incubation of the intact cells was desired, 2 ml of washed packed cells were added to 12 ml of Robinson's medium (6) together with other additions (see "Results").

Livers were excised; washed with 0.9% sodium chloride solution; minced with scissors; mixed with 3 volumes (w/v) of a solution containing 0.35 μm sucrose, 0.025 μm potassium chloride, and 0.004 μm magnesium chloride (7) and homogenized as above.
of the final solution of nucleotides in 0.1 N NaOH was used for determination of the radioactivity (see below). A second aliquot was diluted with water, and the amount of nucleic acids was determined by measuring the absorbance at 260 μg in a Zeiss PMQ II spectrophotometer. An absorbance coefficient of 34.2 mg⁻¹ cm⁻¹ was used (15). Preparation of fractions for the separate determination of RNA and DNA in the remaining portion of the 0.1 N NaOH was as described by Wool (10) (see Reference 14). Ribose and deoxyribose were determined by the methods of Meijbaum (15) and Cieriotti (16), respectively.

In early experiments appropriate samples (see below) were placed in capped stainless steel planchets 1 inch in diameter and ⅛ inch deep, lined with a disk of lens paper (3.26 μg per disk). Lining facilitated even distribution of the material in the planchet. The samples were dried under an infrared lamp and their radioactivity was determined in a model D47 gas flow counter equipped with a Micromil window (150 μg per cm²) supplied by the Nuclear-Chicago Corporation. Elapsed time for the registration of 10⁵ counts was measured for all samples. The counting efficiency in samples of infinite thinness on planchets without lining was 17% of the disintegrations for 14C. The quantities of nucleic acid required for counting satisfied in all instances conditions for infinitely thin application on the planchets. However, it was often necessary to use aliquots from solutions of nucleic acid in either 0.1 N HCl or 0.1 N NaOH and in lined planchets. In order to account for self-absorbance of such samples, the registered counts were first corrected for background and then multiplied by the following empirical factors: for 0.5 and 1.0 ml of 0.1 N HCl, 1.50 and 1.61, respectively.

### Table I

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of tumor (days)</th>
<th>Time after injection (hrs)</th>
<th>Distribution of 14C</th>
<th>Radioactive compound added</th>
<th>Radioactivity in nucleic acid (dpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>4</td>
<td>Ascites cells RNA cpm/mg DNA</td>
<td>ATP</td>
<td>352</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6</td>
<td>Liver DNA cpm/mg</td>
<td>AMP</td>
<td>308</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>Ascites cells RNA cpm/mg DNA</td>
<td>IMP</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>6</td>
<td>Liver DNA cpm/mg</td>
<td>IAD</td>
<td>431</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>6</td>
<td>Ascites cells RNA cpm/mg DNA</td>
<td>IAD</td>
<td>157</td>
</tr>
</tbody>
</table>

Sodium nucleotides were isolated by the method of Barnum and Huseby (8), later modified by Heet and Potter (9). The method was applied essentially as described by Wool (10), the main difference being that 5% cold trichloroacetic acid was used to wash the precipitated proteins six times. An aliquot of this solution was used for determination of its radioactivity (30.2 cpm above background per 0.05 ml). From the rest 3.3 mg of RNA in 3.75 ml of 0.1 N HCI and 1.6 mg of DNA in 1.0 ml of 0.1 N NaOH. Radioactivity determinations in aliquots of the latter solutions gave 38.4 cpm above background per 0.5 ml, and 29.0 cpm above background per 0.5 ml, respectively. Volumes of the final solutions and of plated aliquots were the same as with the same animal were 10.7, 6.0, and 1.5 mg, respectively. Volumes of 5% trichloroacetic acid were collected, and the cells were isolated and washed as described in "Materials and Methods." The packed cells were added to 5 times their volume of 5% trichloroacetic acid and homogenized. The livers of the animals were excised, washed with NaCl, minced with scissors, and homogenized in ice-cold 5% trichloroacetic acid. Nucleic acids were isolated as described in "Materials and Methods." The yield of nucleic acid from ascites cells in Experiment 2 (cited here as an example) was 0.7 mg in 3.0 ml of 0.1 N NaOH. An aliquot of this solution was used for determination of its radioactivity (30.2 cpm above background per 0.05 ml). From the rest were isolated 3.3 mg of RNA in 3.75 ml of 0.1 N HCI and 1.6 mg of DNA in 1.0 ml of 0.1 N NaOH. Radioactivity determinations in aliquots of the latter solutions gave 38.4 cpm above background per 1 ml and 29.0 cpm above background per 0.5 ml, respectively. The yields of nucleic acid, RNA, and DNA from the liver of the same animal were 10.7, 6.0, and 1.5 mg, respectively. Volumes of the final solutions and of plated aliquots were the same as with the ascites cells. All figures were corrected for salt quenching of radioactivity by the solids of the solvents as described in "Materials and Methods."

Incorporation in vivo of 14C from IAD into liver and ascites cell nucleic acids

14C-IAD (5 μmoles, 150,000 cpm) was dissolved in 1 ml of sterile 0.9% NaCl and injected intraperitoneally into mice bearing ascites tumor. At the times indicated in the table, the animals were killed by inhalation of ether, the ascitic fluid was collected, and the cells were isolated and washed as described in "Materials and Methods." The packed cells were added to 5 times their volume of 5% trichloroacetic acid and homogenized. The livers of the animals were excised, washed with NaCl, minced with scissors, and homogenized in 5% trichloroacetic acid. Nucleic acids were isolated as described in "Materials and Methods." The yield of nucleic acid from ascites cells in Experiment 2 (cited here as an example) was 0.7 mg in 3.0 ml of 0.1 N NaOH. An aliquot of this solution was used for determination of its radioactivity (30.2 cpm above background per 0.05 ml). From the rest were isolated 3.3 mg of RNA in 3.75 ml of 0.1 N HCI and 1.6 mg of DNA in 1.0 ml of 0.1 N NaOH. Radioactivity determinations in aliquots of the latter solutions gave 38.4 cpm above background per 1 ml and 29.0 cpm above background per 0.5 ml, respectively. The yields of nucleic acid, RNA, and DNA from the liver of the same animal were 10.7, 6.0, and 1.5 mg, respectively. Volumes of the final solutions and of plated aliquots were the same as with the ascites cells. All figures were corrected for salt quenching of radioactivity by the solids of the solvents as described in "Materials and Methods."
Results of incorporation of radioactivity from \(^1\)C into subcellular fractions of ascites tumor cells

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Radioactive compound added</th>
<th>Radioactivity in fraction (dpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>AMP</td>
<td>400</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>IAD</td>
<td>300 (200)</td>
</tr>
<tr>
<td>600 X g precipitate</td>
<td>AMP</td>
<td>470</td>
</tr>
<tr>
<td>600 X g precipitate</td>
<td>IAD</td>
<td>680 (488)</td>
</tr>
<tr>
<td>105,000 X g precipitate</td>
<td>AMP</td>
<td>90</td>
</tr>
<tr>
<td>105,000 X g precipitate</td>
<td>IAD</td>
<td>570 (385)</td>
</tr>
<tr>
<td>105,000 X g supernatant</td>
<td>AMP</td>
<td>709</td>
</tr>
<tr>
<td>105,000 X g supernatant</td>
<td>IAD</td>
<td>1200 (808)</td>
</tr>
</tbody>
</table>

Fig. 2. Dependence on time of the incorporation of \(^1\)C from labeled 5'-AMP and (5-amino-4-carboxamidomidazole)adenine dinucleoside pyrophosphate (ImAD) into the acid-insoluble fraction of ascites cell homogenates. Incubations were performed as in Table IV, but on a 3-fold scale. At the times indicated on the abscissa, 0.05-ml samples were applied on Whatman No. 1 paper (3.1 X 1.25 cm), treated as indicated in Table IV, and counted in the scintillation counter (see “Materials and Methods”).

RESULTS

Results of an incorporation in vivo of radioactivity from \(^1\)C-labeled IAD are gathered in Table I. Nucleic acids of both ascites cells and liver became labeled. In liver the incorporation was greater but only RNA was labeled. In ascites cells, both components of nucleic acids acquired radioactivity but RNA was labeled to a greater extent than DNA. In Experiment 2 (Table I), 6 hours after injection, 20% of the radioactivity remained in the ascitic fluid and another 20% was excreted in the urine. Determination of diazotizable amines (25) in the urine gave a value of 0.28 pmole. The ratio of the absorbances at 540 and 600 mp of the chromophore in the Bratton and Marshall test was 2.0, indicating that the imidazole was probably

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1. We wish to thank Dr. H. G. Schweiger for performing a number of these fractionations.
in the form of a ribonucleoside (1). Ionophoretic studies (21) in the presence of proper controls, at pH 3.9 and 8.2, revealed two unequal radioactive spots in the urine. The larger of these spots was ultraviolet-quenching (Mineralight SL-2537 lamp) and, on the basis of its movement, it was tentatively identified as inosine. The smaller spot, approximately 20% of total radioactivity in the two spots, gave a positive Bratton and Marshall test. Its movement resembled that of 5-amino-4-carboxamidomidazole ribonucleoside. No further attempt to identify these excretion products was made.

In another experiment, intact washed ascites tumor cells were incubated in vitro in Robinson's medium (6) (see "Materials and Methods"), with 0.7 mM ammonium formate, 0.35 mM UTP, 0.35 mM CTP, and 3.8 μmoles of 14C-labeled IAD (114,000 cpm, total) at 37° for 1 hour with shaking. The cells were then washed three times in the centrifuge with Robinson's medium in the cold, and homogenized with ice-cold 5% trichloroacetic acid. There was no detectable radioactivity in the isolated nucleic acids.

Table II shows the results of a comparative study of the incorporation of radioactivity into nucleic acids in homogenates from 14C-labeled ATP or from AMP, IMP, or IAD. Incorporation was higher in ascites cell homogenates than in liver homogenates. However, in the latter, labeling from IAD was more extensive than from ATP, AMP, or IMP. A similar picture was observed in ascites cell homogenates (Table II). When the latter were fractionated by centrifugation, highest incorporation of radioactivity into nucleic acids from labeled IAD was found in the 105,000 x g supernatant (Table III). With the exception of the whole homogenate, incorporation of radioactivity from IAD was higher than from AMP.

Although isolation of nucleic acids was the method of preference in our studies, it required relatively large quantities of IAD.

### TABLE IV

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentrations</th>
<th>Omissions</th>
<th>Radioactivity in the acid-insoluble fraction</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td>None</td>
<td>1580</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2. Cyanide and iodoacetate</td>
<td>0.5 mm</td>
<td>Phosphoenolpyruvate and pyruvate kinase</td>
<td>1150</td>
<td>28</td>
</tr>
<tr>
<td>3. Aminopterin</td>
<td>0.2 μg, total</td>
<td>Formate and tetrahydrofolute</td>
<td>1120</td>
<td>30</td>
</tr>
<tr>
<td>4. Actinomycin D</td>
<td>2 μg, total</td>
<td>None</td>
<td>1060</td>
<td>34</td>
</tr>
</tbody>
</table>

Fig. 3. Fractionation of an alkaline hydrolysate of RNA from ascites cell whole homogenate after incubation with 14C-IAD. Incubation was conducted as in Table IV, but on a 10-fold scale with homogenate corresponding to 20 mg of protein and 10 μmoles (2.2 x 10⁶ dpm total) of IAD. At the end of the incubation 0.50 ml of 20% trichloroacetic acid was added and the nucleic acids were isolated, hydrolyzed in 0.5 n KOH at 37° for 18 hours (28), acidified with perchloric acid in the cold to remove DNA by centrifugation, and brought to pH 7.0 with KOH. The hydrolysate was centrifuged; to the supernatant solution was added carrier adenosine (0.4 μmole) (24), and it was fractionated on a Dowex 1-X10 (formate form) column. FA corresponds to 30 ml of 0.02 M formic acid (24). G₁, G₂, and G₃ stand for 50 ml of 0.6 M formic acid, 50 ml of 1.33 M formic acid, and 100 ml of 5 M formic acid, respectively (23). The first elution peak (FA) corresponded to ribonucleosides; the others were CMP, AMP, GMP, and UMP, respectively.
and was time-consuming. For this reason, kinetic and other studies were performed by determining the radioactivity in the acid-insoluble fraction of ascites cell homogenates on paper strips. In general, incorporation of radioactivity measured under these conditions from various labeled precursors was higher than when nucleic acids were isolated. However, despite extensive washings of the paper strips with acid, a part of this incorporation should be attributed to trapping (see also Reference 28). This is indicated in Fig. 2. In this experiment, incorporation of radioactivity increased with time from the level of “zero time” measurements, when either IAD or 5’-AMP was used as the labeled precursor. After 60 min, the rate of incorporation from IAD declined (not shown in Fig. 1). No measurements were made after 60 min in the AMP-containing sample.

In an effort to clarify the mechanism of this incorporation, various inhibitors were added in the incubation mixtures. The compounds studied included general metabolic inhibitors (cytochrome c, iodoacetate), which inhibited the formation of ATP in the system; aminopterin, which interfered with folate reductase (29) and consequently with closure of the ring leading to purine formation from 5-amino-4-carboxamidodihydmidazole ribonucleotide (30); and actinomycin D, an inhibitor of nucleic acid bioynthesis (31). It is apparent (see Table IV) that all these substances inhibited incorporation of radioactivity from labeled IAD into the acid-insoluble fraction of ascites cell homogenates to about the same extent.

In another experiment, ascites cell homogenate (30 mg of protein) and 14C-IAD (15 μmoles, 3.3 X 10^6 dpm) were incubated as described in Table IV but on a 20-fold scale of the incubation medium. Labeled nucleic acid (2.8 mg, 1450 dpm) isolated from this experiment was divided into three equal portions and incubated at pH 5 (0.05 M sodium acetate) at 37°C for 15 min with shaking in the presence of either RNase (I) or RNase II, or in the absence of any enzyme (III). During the experimental period 10 and 20% of the total radioactivity and 35 and 44% of the expected absorbance at 260 mμ (corrected for hypochromism (32)) became acid-soluble in Mixtures I and II, respectively.

The nature of the incorporated units was clarified in experiments in which nucleic acids were subjected to alkaline hydrolysis and the mixture was fractionated on a Dowex 1-X10 (formate form) column (Fig. 3). Most of the radioactivity was recovered in the combined fraction of water washings and the eluate of 0.02 M formic acid. It most probably represented adenosine (terminal incorporation). The rest of the radioactivity was recovered from the fractions corresponding to AMP and GMP (Fig. 3). Specific radioactivities of these fractions were the same but the quantity of GMP was approximately twice that of AMP.

**Discussion**

Results reported in this communication are in agreement with previous observations (33, 34), indicating that 5-amino-4-carboxamidodihydrazole or its ribonucleoside may serve as a source of purine moieties incorporated into nucleic acids. However, in our experiments the dinucleoside pyrophosphate rather than the free base or its ribonucleoside was used.

In incubations in vivo of intact Ehrlich ascites tumor cells with labeled IAD, no incorporation of radioactivity into nucleic acids occurred. Although present concepts of cell membrane construction provide for the presence of enzymes capable of degrading complex molecules prior to their uptake in the cell (35), systems degrading dinucleoside pyrophosphates similar to IAD (e.g. NAD or NADH) appear to be absent from the membrane of this strain of Ehrlich ascites tumor cells.

When the IAD was administered in vivo, incorporation of radioactivity was observed in nucleic acids of both the liver and the ascites tumor cells. According to expectations for a nongrowing tissue, there was no incorporation of radioactivity in liver DNA, while in the proliferating ascites cells (36) both DNA and RNA were labeled. However, the specific radioactivity of liver RNA was much higher than the specific radioactivity of either RNA or DNA isolated from Ehrlich ascites cells. A probable explanation would be that, in vivo, the dinucleoside pyrophosphate was first degraded in the liver to units (e.g. the ribonucleosides) for which no problem of permeability through the ascites cell membrane existed. Whether the necessary transformations of IAD or the 5-amino-4-carboxamidodihydrazole ribonucleotide (see below) occurred in this instance in the liver rather than in the ascites cells, it is not possible to decide on the basis of the present evidence.

Part of the administered radioactivity (about 20% of the total) was excreted in the urine during experiments in vivo. This quantity was small in comparison to the extent of degradation and excretion of the histamine adenine dinucleoside pyrophosphate studied earlier in this laboratory (20). The major excretion product in the present studies was most probably labeled iodosine. Excretion apparently occurred after the imidazole moiety in the molecule was transformed (probably intracellularly) to hypoxanthine. Our results indicate that transformations of the 5-amino-4-carboxamidodihydrazole, either at the mononucleotide (30) or at the dinucleotide pyrophosphate (2) level, are possible in both liver and ascites tumor cells. This is evident from results obtained with homogenates from either tissue with the dinucleoside pyrophosphate as the precursor, and from the inhibitory effect of aminopterin upon the time-dependent incorporation of radioactivity from IAD in ascites cell homogenates. The larger quantity of labeled guanylic acid (as compared to AMP) isolated from alkaline hydrolysates of nucleic acid may reflect the favored conversion of 5-amino-4-carboxamidodihydrazole ribonucleoside to guanosine rather than to adenosine (34, 38). This favored conversion was probably due to an inhibition of adenosine deaminase by 5-amino-4-carboxamidodihydrazole ribonucleoside (39). The greater incorporation of radioactivity in the nucleic acids of homogenates of ascites cells from preformed purine nucleotides (ATP), as compared to the 5-amino-4-carboxamidodihydrazole dinucleoside pyrophosphate, probably reflects the tendency of cells in vivo to utilize pre-existing purine nucleotides (33) (see also Reference 40). It would be expected that this reluctance to biosynthesize purines de novo would be accentuated in cell-free systems.

Note worthy are results obtained with liver homogenates (Table II). Although IAD is metabolically several steps behind ATP and two or three steps behind IMP as a building stone of nucleic acids, incorporation of radioactivity was higher from IAD into liver RNA. Similar results were observed with various fractions obtained by centrifugation of ascites cell homogenates. Thus, incorporation of radioactivity from IAD in the acid-insoluble fraction of the 105,000 X g precipitate of the homogenate, and in the supernatant of this centrifugation, was higher than incorporation from AMP. This pointed towards a predominantly terminal type of incorporation of the
Radioactivity (24, 41) from IAD in the nucleic acids of cytoplasmic fractions. This assumption was verified experimentally (see “Results”). Favorable terminal incorporation of nucleotide units in nucleic acids from the dinucleoside pyrophosphate is difficult to reconcile with presently accepted pathways (i.e. involving hydrolysis of the dinucleoside pyrophosphate into mononucleotides and subsequent formation of the nucleoside triphosphates as suggested by Revel and Mandel (42)) (see also References 7, 43, 44). However, much more experimental evidence is required in order to postulate (or exclude) a direct incorporation from the dinucleoside pyrophosphate level at the expense of the free energy of hydrolysis of the pyrophosphate bond in IAD.

SUMMARY

Radioactivity from (5-amino-4-carboxamido-2-14C-imidazole)-adenine dinucleoside pyrophosphate was administered intraperitoneally into mice bearing Ehrlich ascites tumor. This radioactivity is found to be incorporated into both ribo- and deoxyribonucleic acid of ascites cells but only into ribonucleic acid of liver cells. Only a small fraction of the total radioactivity was excreted in the urine of the animal during the experimental period (6 hours). The main excretion product was tentatively identified as inosine.

In incubations in vitro there was no incorporation of radioactivity from the dinucleoside pyrophosphate into the nucleic acids of intact ascites cells.

In whole homogenates of mouse liver, incorporation of radioactivity into nucleic acids was greater from the dinucleoside pyrophosphate than from 8-14C-adenosine 5’-phosphate, or adenosine triphosphate. In whole homogenates of ascites tumor cells, the incorporation from labeled adenosine triphosphate was greater than from labeled dinucleoside pyrophosphate than from 8-14C-labeled inosine 5’-phosphate, or actinomycin D partially inhibited incorporation of radioactivity into nucleic acids of cytoplasmic fractions. This assumption was verified experimentally involving hydrolysis of the dinucleoside pyrophosphate into mononucleotides and subsequent formation of the nucleoside triphosphates as suggested by Revel and Mandel (42). The presence of cyanide plus iodoacetate or of aminopterin or actinomycin D partially inhibited incorporation of radioactivity from the dinucleoside pyrophosphate into the acid-insoluble fraction of ascites cell homogenates.

Fractionation of an alkaline hydrolyzate of the ribonucleic acids isolated from ascites cells showed that terminal incorporation prevailed. However, in all instances, nonterminal incorporation was also observed.

REFERENCES

Incorporation of Radioactivity from (5-Amino-4-carboxamido-2-\(^{14}\)C-imidazole) adenine Dinucleoside Pyrophosphate into Nucleic Acids
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