COMPARTMENTATION OF INTRACELLULAR NUCLEOTIDES IN MAMMALIAN CELLS

Authors: **James D. Moyer**
Laboratory of Medicinal Chemistry and Pharmacology
National Cancer Institute
Bethesda, Maryland

J. Frank Henderson
Cancer Research Group and Department of Biochemistry
University of Alberta
Edmonton, Alberta, Canada

Referee: Mary Ellen Jones
Department of Biochemistry and Nutrition
University of North Carolina
Chapel Hill, North Carolina

I. INTRODUCTION

Purine and pyrimidine nucleotides are involved in almost every area of cellular metabolism, and alterations in nucleotide concentrations have been related to numerous pathological conditions; in addition, many drugs have been developed to affect one or another aspect of nucleotide metabolism. As a consequence of the important role of nucleotides in biochemistry, pathology, and pharmacology, the measurement of nucleotide concentrations (or nucleotide "pools") has assumed considerable importance in many areas of biomedical research. Such measurements most often are made (1) to establish the specific activity of a precursor nucleotide pool in order to calculate the rate of synthesis of a product; or (2) to attempt to relate the intracellular concentration of a nucleotide with the rate of some process that requires this nucleotide.

Both historically and at the practical level of laboratory investigation, it tends to be assumed that the nucleotide pools which are measured are homogeneous, and it would certainly be most convenient for this to be so. Increasingly, however, evidence of a variety of sorts is appearing that suggests or even clearly indicates, that purine and pyrimidine nucleotide pools are heterogeneous or compartmentalized rather than homogeneous.

Krebs[1] has broadly defined compartmentation as the "sub-division of an organism, or an organ, or of a cell into areas which differ qualitatively or quantitatively in respect to their chemical constituents." This review, however, will be concerned more specifically with intracellular compartmentation, and will therefore mainly consider studies using preparations of a single cell type; intercellular compartmentation of nucleotides within animal tissues, though also of importance, will be considered here only in passing. Most studies of nucleotide compartmentation have employed mammalian cells, and hence this review emphasizes such biological systems; however, results obtained using other types of eukaryotic cells are mentioned when appropriate.

Evidence for compartmentation of purine and pyrimidine nucleotide pools may be said to be "direct" when such inhomogeneity can be ascribed to concrete cytological entities (e.g., mitochondria) or molecular species (e.g., proteins that bind nucleotides very tightly, or multienzyme complexes in which "channeling" may occur). Evidence for compartmen-

tation is considered "indirect" if it is based on rates of radioactive precursor metabolism or unexpected changes in measured nucleotide concentrations, but cannot definitively be associated with a cytological or molecular entity. The review will be organized along these lines.

Approaches to the subject of purine and pyrimidine nucleotide compartmentation vary widely. Sometimes there is a tendency to ignore the possibility of compartmentation, and sometimes all apparently discordant data are ascribed to it. Only occasionally is there an explicit attempt to investigate whether a pool is homogeneous or not at the beginning of a study, and this subject is still at an early stage of development with respect to understanding the consequences of various kinds of compartmentation.

The purpose of this review is to summarize the existing evidence for compartmentation of purine and pyrimidine nucleotide pools and to distinguish among the various bases and types of evidence, discuss the limitations of this evidence, and to consider the implications of compartmentation for the interpretation of nucleotide pool measurements. Several monographs concerned with compartmentation at a more general level have been published,[2,3] and a review of techniques for the investigation of intracellular compartmentation has been published.[4] The compartmentation of pyrimidine nucleotides has been reviewed by Keppler.[5]

II. DIRECT EVIDENCE FOR COMPARTMENTATION OF NUCLEOTIDES

Nucleotide compartmentation is most easily conceived of when it is associated with cytological entities such as organelles, or with molecular species such as individual proteins or multienzyme complexes. At least in theory such compartmentation is also the most amenable to experimental investigation; if adequate rapid separation of the organelles or proteins can be achieved, then differences in nucleotide concentrations and their specific activities can be measured directly.

A. Compartmentation in Cellular Organelles

1. Mitochondria

Mitochondria are separated from the cytoplasm by a double membrane and possess independent enzymes for nucleic acid synthesis. It is therefore not surprising that many studies have shown that the nucleotide pools of mitochondria form a separate compartment. It has been reported, for example, that ATP/ADP ratios are different in mitochondria than in the whole cell, and that the pools used for mitochondrial DNA synthesis have distinctive properties. Indeed, this compartmentation is the most clearly documented of all forms reported, and space permits the citation of only selected references.

a. Nucleotide Coenzymes of Energy Metabolism

The central role of mitochondria in energy metabolism has inspired a number of studies comparing ATP and ADP concentrations in mitochondria and cytoplasm.[6] This question has been examined in rat liver,[7-10] isolated liver cells,[4,11-13] perfused liver,[14] perfused heart,[15] and myocardial cells.[16] In most studies a higher ATP/ADP ratio has been observed in cytoplasm than in the mitochondria, but contrary reports have also appeared.[4,9] In addition to the differences in this ratio, the absolute concentration of ATP per microliter of water appears to be higher in mitochondria than in cytoplasm.[12,13] Fewer studies have been made of nucleotides other than the adenylates, however, guanine nucleotides have been reported to be present at twofold higher concentration in mitochondria than in cytoplasm.[11] A recent summary of work concerning guanine nucleotides in mitochondria has been published by Kleineke et al.[11]

Most measurements of ATP and ADP in the mitochondrial matrix require a rapid separation of this organelle from the cytoplasm, and in view of its small dimensions and high levels

of adenylate metabolizing enzymes it is difficult to be certain that their isolation is rapid enough to prevent major changes in nucleotide concentrations (for a more detailed discussion of these difficulties see the article by Veech[6]). The presence of a very active bidirectional carrier for the transport of ATP and ADP across the mitochondrial membrane might suggest that any compartmentation of these nucleotides would be in the form of a concentration differential,[17] because the specific activities of cytoplasmic and mitochondrial adenylates should rapidly equilibriate. For example, Hartung et al.[18] found that a complete equilibrium of inorganic phosphate and ADP between the medium and mitochondria occurred in less than 7 sec at 25°. The exchange of adenine nucleotides between the mitochondria and the cytoplasm is discussed in detail by Klingenberg.[19]

b. Precursors of DNA and RNA

Berk and Clayton[20] have demonstrated convincingly that the dTTP pool used for synthesis of mitochondrial DNA is distinct from the total cellular dTTP of mouse L cells. They found that in mutant cells lacking cytoplasmic thymidine kinase, but expressing the mitochondrial form of this enzyme, [^{3}H]thymidine labeled exclusively mitochondrial DNA. This indicated that the dTTP formed from the thymidine was not available for nuclear DNA synthesis, which proceeded by use of dTTP synthesized *de novo*. Further evidence of this compartmentation was obtained by Bogenhagen and Clayton,[21] who found that nuclear DNA synthesis in thymidine kinase-deficient cells was extremely sensitive to 5-fluorouracil deoxyribonucleoside while mitochondrial DNA synthesis was only moderately sensitive if low concentrations of thymidine were provided.

Bestwick et al.[22] have also provided evidence for separate mitochondrial pools of deoxyribonucleoside triphosphates in HeLa cells. These authors found that the mitochondrial pools responded quite differently than total cellular pools (predominantly cytoplasmic) to treatment with methotrexate or 5-fluorodeoxyuridine. Specifically, methotrexate caused marked contraction of total cellular dTTP and dGTP pools, but these pools increased in the mitochondrial compartment during the treatment.

Although the evidence for a degree of compartmentation of mitochondrial deoxyribonucleoside triphosphate pools seems convincing, it is likely that some exchange of these nucleotides does occur between mitochondria and cytoplasm, since studies in vitro with isolated rat liver mitochondria have indicated a ready utilization of exogenous deoxynucleoside triphosphates for the synthesis of mitochondrial DNA.[23]

Soeiro and Ehrenfeld[24] examined the labeling of mitochondrial RNA by [^{3}H]uridine and found evidence for an RNA precursor pool distinct from the pool used for nuclear RNA synthesis. Further studies indicated that the pool drawn upon for mitochondrial RNA synthesis was also distinct from that used for poliovirus RNA synthesis, a process which occurs in the cytoplasm.

c. Carbamyl Phosphate

Finally, though not really a nucleotide, the compartmentation of carbamyl phosphate in relation to the process of pyrimidine nucleotide biosynthesis may be mentioned at this point.

Studies in *Neurospora* by Davis and his colleagues[25] have indicated that the carbamyl phosphate pool formed by carbamyl phosphate synthetase A, an enzyme of arginine synthesis, and the pool of carbamyl phosphate formed by carbamyl phosphate synthetase B, an enzyme of pyrimidine biosynthesis, do not mix freely, but rather are compartmentalized to a considerable extent. Thus mutants deficient in carbamyl phosphate synthetase B require uridine for growth although the concentration of carbamyl phosphate measured in these cells is only slightly lower than in cells of the wild type. This compartmentation was attributed in part to sequestration of the carbamyl phosphate produced by carbamyl phosphate synthetase A in the mitochondria.

Mammalian cells also contain two separate carbamyl phosphate synthetases. One, designated carbamyl synthetase I, is found in mitochondria and functions in arginine synthesis and the urea cycle. The other, designated carbamyl synthetase II, is located in the cytoplasm as a component of a multienzyme complex responsible for the early steps of pyrimidine synthesis *de novo*.

Recent studies with both liver slices[26] and isolated hepatocytes,[27] however, have revealed that in liver the carbamyl phosphate produced by the mitochondrial enzyme (type I) is available for pyrimidine biosynthesis. In fact at physiological concentrations of ammonia the bulk of the carbamyl phosphate used in pyrimidine synthesis was of mitochondrial origin. Thus if separate pools of carbamyl phosphate are present in hepatocytes there must be a significant degree of mixing.

2. Granules

A second type of organelle which is associated with intracellular compartmentation of nucleotides is the granules which occur in certain types of cells.

For example, a metabolically unavailable pool of adenine nucleotides is found in the chromaffin granules of the adrenal medula. A high percentage of the total ATP present in this tissue is present in granules containing adrenaline and noradrenaline,[28,29] and this is not available to cellular enzymes and is only released upon lysis of the granules.

Platelets also contain a pool of ATP and ADP which is metabolically inert, at least on a scale of hours.[30,31] This portion of the nucleotide pool, approximately 65% of the total, is stored in the dense granules. In these granules the ATP and ADP, in a 2:3 ratio, are present as insoluble calcium complexes.

3. Nuclei

The possibility that nucleotides are compartmentalized within nuclei has been considered both with respect to the purine and pyrimidine ribonucleoside triphosphate precursors of nuclear RNA synthesis, and to the corresponding deoxyribonucleoside triphosphate precursors of DNA synthesis. Numerous reports of equal distribution of small molecules and soluble enzymes across the nuclear membrane, as well as the presence of large (10 nm) pores, suggest that the nuclear membrane is unlikely to serve as an effective barrier to nucleotides, however. A summary of evidence concerning nuclear-cytoplasmic compartmentation has been published by Siebert.[32] The possibility remains, however, that very rapid rates of utilization of ribo- or deoxynucleoside triphosphates in the nucleus might give rise to concentration gradients. Definitive evidence on this question awaits the development of methods for the very rapid and quantitative separation of the nucleus and cytoplasm; at present, aqueous methods of nuclear isolation lead to loss of nucleotides, and nonaqueous methods give low yields. In the meantime the various types of evidence that might suggest nuclear compartmentation of nucleotides is summarized below.

a. Ribonucleotides

The distribution and transport of ribonucleoside triphosphates between the nucleus and the cytoplasm has not been extensively examined. Fractionation of liver nuclei have indicated that concentrations of ATP, ADP, and AMP in nuclei and cytoplasm are about equal, and a similar response to anoxia is seen in both compartments.[33] In addition, the specific activity of ATP after [^{14}C]formate labeling was found to be similar in both compartments.[34] These studies suggest a rapid equilibrium between nuclear and cytoplasmic ATP pools, although it is difficult to assess the extent of equilibration which might occur during nuclear isolation. Studies are clearly required to determine whether or not nucleotides can rapidly transverse the nuclear membrane. If such flux is slow relative to utilization rates for RNA synthesis, a type of compartmentation might result. If movement is rapid across this membrane,

however, it would seem that any apparent nuclear compartmentation would require another mechanism, such as channeling in a multienzyme complex.

If equilibration of nucleotides across the nuclear membrane is slow relative to rates of synthesis and utilization, then various enzyme activities in the two compartments would be critical in establishing differential concentrations and specific activities during labeling. Little information in the literature is available concerning the location of the nucleoside kinases involved in salvage synthesis or the enzymes of *de novo* synthesis. At least one step of *de novo* pyrimidine synthesis takes place in mitochondria. The most common tracer used to follow pyrimidine synthesis *de novo*, however, is orotic acid which enters the pathway at a subsequent step. Further studies on the localization of enzymes of nucleotide metabolism would clearly be useful.

b. Deoxyribonucleotides

The question of distinct nuclear and cytoplasmic compartments of deoxynucleoside triphosphates has been approached by means of cell fractionation[31,35,36] and autoradiography.[37,38]

Skoog and Bjursell[35] used a nonaqueous fractionation method to prepare nuclei from Chinese hamster ovary (CHO) cells. Their results supported a compartmentation of nucleotides since dTTP appeared to be more concentrated in the nuclear fraction than in cytoplasm. In contrast, dATP, dCTP, and dGTP were more equally distributed or even present in lower concentrations in nuclei than in cytoplasm. Furthermore, hydroxyurea had divergent effects on whole cell and nuclear dTTP pools of S-phase CHO cells: the pools increased 70% as measured in whole cell extracts, whereas a small (18%) decrease in dTTP concentrations were observed in the nuclear fraction. Thymidine, in contrast, had generally similar effects on pools measured in both nuclear and whole cell extracts. Although these data indicated a separate nuclear compartment at least of dTTP, the very low (4 to 5%) recovery of nuclei (as estimated from DNA) makes it impossible to exclude large differences in recoveries of nucleotides in different fractions. In addition, it is impossible to exclude redistribution of nucleotide during extraction and separation on the basis of available information on this procedure, though this seems unlikely.

Bestwick and Mathews[39] have also found unequal distribution of deoxynucleoside triphosphates between nuclei and cytoplasm in HeLa cells. In these cells the ratio of cytoplasmic pool size to nuclear pool size ranged from 9 for dATP to 0.8 for dCTP. Thus in contrast to the results in CHO cells, dCTP seemed to be the nucleotide specifically concentrated in the nuclei.

Additional support for intranuclear localization of dTTP has been presented by Adams and co-workers.[37,38] Mouse fibroblasts were labeled with [^{3}H]-thymidine and then fixed for autoradiography. Acid-extractable radioactivity appeared as a "halo" around the nucleus rather than generally throughout the cytoplasm, suggesting a nuclear location for dTTP. Without quantitation of recoveries and evidence concerning the chemical nature of the material giving rise to the "halo", however, this evidence cannot be taken as conclusive.

B. Nucleotides Tightly Bound to Proteins

A second basis of compartmentation is the very tight binding of nucleotides to proteins or other cellular components. If such binding was tight and release to the free form in cell water was slow, the existence of such a compartment could result in an underestimation of the specific activity of the free nucleotide pool during short-term labeling studies with radioactive nucleosides or bases. Binding to protein would lead to an overestimate of the concentration of nucleotides available to enzymes. For most enzymes utilizing ribonucleoside triphosphates for substrates this binding is rapidly reversible, as indicated by high turnover numbers. However, a number of proteins have been found to exhibit a tight binding of nucleotides. Among the most important are tubulin, which tightly binds GTP;[40] actin, which

binds ADP;[6,41] hemoglobin which binds ATP,[42] and an unidentified structural protein of mitochondria.[43]

Tight binding of this sort would affect the apparent concentration of nucleotide available to enzymes, and if the exchange was slow relative to utilization, the apparent specific activity of nucleotides undergoing polymerization to form RNA. In muscle about 5% of the total adenine nucleotide pool apparently is bound as ADP to actin,[41] while considered from another point of view, Seraydarian et al.[44] have suggested that 80% of the ADP in muscle is bound to actin; these figures are equivalent. In cardiac muscle binding to actin has been shown to significantly lower the free concentration of ADP;[15,44] however, this binding is not a significant factor in liver cells.[45] Kinetic data by Saks et al.[46] have provided evidence for binding of ADP by cardiac myofibrillar preparations; this pool of ADP seems to be selectively rephosphorylated by creatine kinase. In contrast Gankema et al.[47] performed binding studies which suggested that 30% of the ADP present in liver cytoplasm could be bound, although ATP was not significantly bound. Studies with human red cells indicated that only 40% of the adenine nucleotides were readily available for metabolism, the remainder being bound and not equilibrated with free nucleotides even after 5 hr of incubation.[42] Thus in specialized cells such as muscle and erythrocytes, where a high concentration of a particular protein is present, binding may be significant.

In Ehrlich ascites cells almost all the ATP appears to be available for metabolism. Both Yushok[48] and Lomax et al.[49] have reported that addition of 2-deoxyglucose could deplete 86% of the ATP in these cells in 5 min and (under anaerobic conditions) 98% was depleted in 30 min. Thus in these cells essentially all ATP is accessible to dephosphorylating enzymes within 30 min, but a fraction of ATP still may be bound but released at rates too rapid to detect in this manner.

C. Channeling Via Multienzyme Complexes

A third possible basis for nucleotide compartmentation is the existence of multienzyme complexes — if there is "channeling" of nucleotide substrates, that is, when substrates go from one enzyme to another without complete mixing with the total intracellular pool of the same nucleotide substrate.

1. Pyrimidine Biosynthesis

In mammalian cells pyrimidine synthesis *de novo* is performed by two multienzyme complexes designated *pyr* 1-3 and *pyr* 5-6 (see Jones[50] for a review). Kinetic analysis with purified enzyme complexes have provided convincing evidence for channeling of carbamyl aspartate and carbamyl phosphate by *pyr* 1-3.[51-53] This channeling is not strict, however, and both release of intermediates from the complex and competition by added substrate have been observed. The complex from rat ascites tumor cells, for example, appears to channel about 11% of the carbamyl phosphate formed.[53]

Channeling of orotidylate by the second enzyme complex of pyrimidine synthesis (*pyr* 5-6) has been demonstrated with enzymes from Ehrlich ascites cells[54,55] and from protozoa.[56] As with the first complex the channeling is not strict and utilization of added orotidylate has been observed.

The extent of channeling of biosynthetic intermediates for pyrimidine biosynthesis in intact cells is unclear at this time, but should be amenable to investigation by the use of permeabilized cells.

2. Deoxyribonucleotides

A second example of channeling has been reported to occur in relation to certain aspects of deoxyribonucleoside triphosphate metabolism, in which multienzyme complexes provide a basis for a compartment which is separate from the total pool of deoxyribonucleoside

triphosphates, since they can be metabolized and incorporated into DNA without mixing with the soluble pool of these nucleotides. Complexes of the enzymes producing deoxynucleoside triphosphates have been reported in T4 phage-infected *Escherichia coli* cells,[57] Novikoff hepatoma cells, Chinese hamster embryo fibroblasts (CHEF),[58,59] and human lymphoblastoid cells.[60] For this review only the evidence from studies with mammalian cells will be considered.

Prem veer Reddy and Pardee[61] reported that a complex of DNA polymerase, thymidine kinase, dihydrofolate reductase, nucleoside diphosphate kinase, and ribonucleotide reductase (called "replitase"), is present in the nuclei of S phase, but not G_0 phase CHEF cells. Furthermore, in cells made permeable to nucleotides and incubated in the presence of dithiothreitol, [^{3}H]CDP was more rapidly incorporated into DNA than was [^{3}H]dTTP, although dTTP is the more proximal precursor. Further evidence for such channeling was obtained with permeabilized cells: the rate of incorporation of [^{14}C]CDP into DNA was not inhibited by the presence of excess nonradioactive dCTP.[61] Furthermore, in extracts containing the putative replitase CDP was apparently incorporated into DNA prior to release of deoxycytidine nucleotides to the incubation medium.[59] These results have been called into serious question, however, and it has been suggested that the [^{14}C]CDP was incorporated into RNA rather than DNA in these experiments.[62]

It has also been suggested that there are two pools of dTTP in human lymphocytes, with one pool fed preferentially by *de novo* synthesis and used preferentially for DNA synthesis.[63,64] An extract from a human lymphoblastoid cell line has been prepared by a gentle lysis procedure, that can catalyze the incorporation of dTTP, dTMP, dUMP, and thymidine into DNA at comparable rates. When the more distal substrates for DNA polymerase are used, the concentration of free dTTP generated in the reaction mixture is well below that required for an equal rate of DNA synthesis when only dTTP is added as a source of thymine nucleotides.[60,64] A complex capable of incorporating dTTP, dTMP, or thymidine into DNA was separated from smaller proteins by gel filtration.[65] More rigorous kinetic studies, particularly studies of the competition between free dTTP and that formed in the complex, are necessary to indicate the extent of channeling.

More complete data are required for an understanding of nucleotide channeling in DNA synthesis, including the identification of the enzymes involved and measurement of the "tightness" of channeling, i.e., the probability of loss from the complex before incorporation and the ability of free nucleotide to compete. It is clear, however, that channeling in such complexes is not strict because thymidine and deoxycytidine are excellent precursors of DNA in mammalian cells, thus a ribonucleotide reductase step in the complex is not obligatory.

III. INDIRECT EVIDENCE FOR COMPARTMENTATION OF NUCLEOTIDES

A considerable body of data has accumulated on the incorporation of radioactive precursors into soluble ribonucleotides and RNA, and into soluble deoxyribonucleotides and DNA, which have been interpreted in terms of nucleotide compartmentation. In most of the systems studied, however, the cytological or molecular basis of such compartmentation is either not known at all or is not well established. In addition, certain studies of the metabolism of cyclic nucleotides have been interpreted in terms of compartmentation, again with no definitive basis being known.

A. Kinetics of Radioactive Precursor Incorporation

1. Compartmentation of RNA Precursors

A frequently proposed form of compartmentation concerns the ribonucleoside triphosphates which serve as precursors for RNA synthesis. It has been reported that pools of UTP and ATP distinct from the total cellular pools serve as specific precursors for RNA in several

types of eukaryotic cells. In addition, several reports suggest that the pool of UTP serving as precursors to HnRNA may be distinct from that serving as precursor to pre-rRNA. The evidence for both forms of compartmentation are summarized in this section.

The evidence for compartmentation of RNA precursors is usually kinetic data showing that the specific activity of the total ribonucleoside triphosphate precursor pool does not correlate with the rate of incorporation of precursor into RNA. For example, Plagemann[66,67] found that incorporation of labeled uridine into RNA did not seem to proceed via the total cellular UTP pool; a number of criteria were considered: (1) prior twofold expansion of the UTP pool had no effect on subsequent incorporation of [^{14}C]uridine into RNA, although the specific activity of the total intracellular UTP pool was reduced; (2) when [^{3}H]uridine was removed from the medium, net incorporation of label into RNA fell sharply, although total cellular UTP pools remained highly labeled for some time; (3) when mengovirus-infected hepatoma cells were grown in the presence of [^{3}H]uridine the incorporation of labeled uridine into mengovirus RNA (synthesized in the cytoplasm) continued at a high rate after removal of [^{3}H]uridine from the medium, whereas incorporation of label into cellular RNA decreased sharply; and (4) incorporation of label from [^{3}H]uridine into RNA was linear within 10 min of addition while the amount of label in UTP increased for at least 2 hr. Plagemann concluded that RNA is synthesized from a pool of UTP distinct from the total cellular UTP pool.

Similar but less extensive experiments have suggested a compartmentation of the UTP pool in mouse embryo cells,[68] murine lymphoma cells,[69] and Chang liver cells.[70] In addition, Khym et al.[71] studied the effect of prior UTP pool expansion on [^{3}H]uridine incorporation in a number of cell lines and found evidence for a degree of compartmentation in Novikoff hepatoma cells (in agreement with Plagemann's results) and HeLa cells. In six other cell lines the authors saw no evidence of UTP pool compartmentation, although these negative results are complicated by decreased uptake of the nucleoside in the cultures with expanded UTP pools.

An important complication of short-term precursor incorporation studies is the degradation of a fraction of the newly synthesized RNA with return of labeled nucleotide to the nucleotide pool. It seems possible that this phenomenon could account for the observed disagreement of specific activity with incorporation which is the basis of points (1) and (2) given above. Thus an increased rate of return of label from RNA to acid soluble pools could compensate for the increased rate of incorporation of label into RNA from UTP. An unambiguous dissection of these two processes has yet to be made.

The difficulties of correcting for return of label to acid-soluble pools can be obviated by several approaches, including measurement of only stable RNA species or long-term incubations. In such an approach Falkenthal and Lengyel[72] examined the rate of labeling of tRNA by [^{3}H]uridine in *Drosophila* cell cultures. By comparing the rate of accumulation of label in tRNA and the rate of accumulation of tRNA by exponentially growing cultures, they calculated the apparent specific activity of the pool of UTP used for tRNA synthesis. They found that this pool had a specific activity about twofold higher than the total UTP pool measured in the cell cultures. This result is compatible with the presence of a more rapidly labeled pool for RNA synthesis as proposed by Plagemann and others. Because the calculation requires many measured values however, the accumulated errors may render this apparent twofold difference in specific activity insignificant.

Particularly strong evidence for a compartmentalized pool of UTP involved in RNA synthesis comes from the studies of Wiegers et al.[73,74] Uridine in the medium of HeLa cell cultures was maintained at a constant specific activity for more than four cell doublings. Although the specific activity of cellular UTP pools rapidly (approximately 50 hr) plateaued at the same value as did that of the added uridine, the specific activity of uracil in rRNA rose only to half this value and did not further approach the level of the soluble pools over the subsequent 50 hr. By contrast, the specific activity of uracil in mRNA became nearly

equal to that of the UTP pool. This strongly suggested that a pool other than the total cellular UTP pool served rRNA synthesis. Similar results were obtained for the CTP pool. By contrast, somewhat similar studies with mouse L cells[75] and chick fibroblasts[76] have indicated that RNA is synthesized from the total cellular ATP pool. In addition, total cellular GTP pools appear to serve RNA synthesis in *Xenopus* oocytes.[77]

Genchev[78,79] has invoked compartmentation of UTP pools to explain unexpected labeling patterns in Ehrlich ascites cells. UTP pools were labeled with two different radioactive precursors: orotate and uridine. The apparent rate of RNA synthesis was calculated by measuring the incorporation of [^{14}C] and correcting for the average specific activity of the UTP pool during the period of incorporation. The two methods of labeling gave results differing by more than tenfold, with uridine the more effective at labeling RNA. Genchev concluded that the precursors were entering different UTP pools and that the pool selectively labeled by uridine was used for RNA synthesis.

These studies need to be interpreted in the light of observations[80] that orotate entry into these cells is very slow, and limited by membrane permeability; in addition, high concentrations of orotate inhibit earlier steps of the pathway of pyrimidine biosynthesis *de novo*.[81] Thus, measurement of intracellular concentrations of orotate would have been helpful. Finally, because of the low rate of utilization of this precursor, measurements of uridine and orotate incorporation had to be carried out over different time periods. Nevertheless, it is not certain that these concerns invalidate Genchev's conclusions.

Losman and Harley[82] also labeled the UTP pool of rat hepatoma cells simultaneously with [^{3}H]orotate and [^{14}C]uridine. The ^{14}C to ^{3}H ratio was identical in the UTP pool and the UMP of RNA, implying that the RNA was synthesized from a pool of UTP with the same characteristics as the total cellular pool. However, the ratio of ^{14}C to ^{3}H in CTP and CMP in RNA was much higher. Since CTP is synthesized from UTP these data suggested that a compartment of the UTP, selectively labeled by uridine, served as a precursor of CTP.

Evidence against separate UTP pools for RNA synthesis has been provided by Puckett and Darnell.[83] The "specific U to C ratio" of RNA and acid-soluble pools were measured in HeLa cells incubated with [^{3}H]uridine. This value was calculated as the ratio of [^{3}H]UMP and [^{3}H]CMP in RNA corrected for the mass of each in the RNA. They found that this ratio was identical in HnRNA, nucleosomal RNA, and the acid-soluble pools, implying that the total UTP and CTP pools served as precursors for both species of RNA.

The question of compartmentation of RNA precursors thus seems unresolved, with considerable evidence supporting the existence of compartmentation at least in some cell lines. One possible mechanism for such compartmentation, often suggested, would be distinct pools separated by the nuclear membrane; however, as discussed above, direct evidence for distinct nuclear pools of nucleotides has not yet been forthcoming. Such pools could be either totally independent or simply slowly equilibrating. Indeed, studies with an RNA virus replicating in the cytoplasm showed different labeling kinetics than those of the cellular RNA synthesized in the cytoplasm,[66,67,84] although such compartmentation was not apparent in all studies.[24]

In addition to the evidence that total RNA is synthesized from a distinct compartment of the nucleotide pool, some authors have suggested that specific classes of RNA are derived from separate compartments. An early examination of this possibility in HeLa cells was made by comparing the approach to equilibrium of the specific activity of UMP and CMP in pre-rRNA and HnRNA.[83] By this criterion these two species appeared to be synthesized from a single pool. Birch and Turnock[85] also found that tRNA and rRNA appeared to be synthesized from a common pool of nucleoside triphosphates in Chinese hamster ovary cells.

In contrast, when HeLa cell UTP pools were labeled to a constant specific activity by [^{3}H]uridine for several generations, the specific activity of UMP in mRNA reached the same specific activity as the UTP pool, while the specific activity of UMP in 28S RNA achieved

only one half the expected specific activity.[74] The authors suggested that the rRNA was synthesized from a small compartment of UTP selectively fed by *de novo* synthesis. Similar studies by Birch and Turnock[85] with CHO cells, however, showed that tRNA and rRNA were synthesized from the same pyrimidine nucleotide pool.

Further evidence for separate UTP pools serving rRNA and HnRNA was obtained when Ehrlich ascites cells were labeled by [^{14}C]orotate or [^{14}C]uridine.[78,79] Although both precursors are incorporated into RNA only after conversion to UTP, and thus presumably via a common pool, they gave markedly different labeling patterns. Uridine labeled rRNA about 10% more than HnRNA, while HnRNA was labeled more than twice as heavily by orotate than was rRNA. Although this study also points to compartmentation, it seems to contradict an earlier study in that this report indicates rRNA is selectively labeled by the salvage of preformed uridine.

Since all three classes of RNA, although synthesized by different polymerases, are produced in the nucleus, this apparent compartmentation of precursors for the different types of RNA cannot be explained simply by two pools separated by the nuclear membrane. More complex forms of compartmentation perhaps involving channeling in multienzyme complexes could be invoked, but no evidence supporting such hypotheses is available.

2. Compartmentation of DNA Precursors

Several investigators have invoked compartmentation of deoxyribonucleoside triphosphates to explain apparently anomolous kinetics of incorporation of labeled thymidine into DNA. Thus Fridland[86] concluded that DNA was synthesized from a distinct compartment of the cellular dTTP pool of human lymphoblast cells on the basis of two arguments. First, upon incubation with [^{3}H]thymidine, the rate of incorporation of label into DNA became constant within 4 min although the specific activity of the dTTP pool continued to rise for at least 20 min. Secondly, when dTTP pools were labeled with [^{3}H]deoxyuridine to the same specific activity as was obtained with [^{3}H]thymidine, the incorporation into DNA of radioactivity originating from deoxyuridine was twice as rapid. He concluded that a special compartment of the dTTP pool served as the precursors for DNA synthesis and that this compartment was selectively labeled by [^{3}H]deoxyuridine.

Mathews[87,88] found that extracellular [^{3}H]thymidine labeled DNA of sea urchin eggs more rapidly than would be expected, as the eggs had very large intracellular dTTP pools; he suggested that the internal pools were bypassed possibly because these pools were compartmentalized. Unfortunately, since the kinetics of incorporation into acid soluble nucleotides were not reported, this argument is not conclusive.

Kinetic studies in mouse L cells labeled with [^{3}H]thymidine were consistent with a single cellular dTTP pool which serves as the precursor for DNA.[89] However, this report also lacked documentation of the kinetics of incorporation of label into the acid-soluble dTTP pool.

Two reports have suggested that dTTP pools are compartmentalized in HeLa cells.[90,91] Baumunk and Friedman[91] found that inhibition of dTTP synthesis by 5-fluorodeoxyuridine or amethopterin led to marked inhibition of DNA synthesis (93%) while dTTP pools were only modestly reduced (<20%). This was interpreted as evidence that a large pool of dTTP exists that is not available for DNA synthesis.

Nicander and Reichard[92] have recently examined the incorporation of labeled cytidine, thymidine, and deoxycytidine into DNA. The simple first-order decay of the dTTP pool on removal of labeled thymidine, as well as the calculated rates when the pool was manipulated, were consistent with a single pool of dTTP in 3T6 cells. In contrast, results with cytidine showed this nucleoside to be more rapidly incorporated into DNA than expected from the specific activity of dCTP achieved. The authors conclude that cytidine labeled a different pool of dCTP than did deoxycytidine and that the pool labeled by cytidine was more directly

related to DNA synthesis. This finding is consistent with a degree of channeling of CDP into DNA as proposed by Prem veer Reddy and his co-workers, but is not evidence for strict channeling in the replitase. An alternative explanation would be selective labeling of a cytoplasmic dCTP pool by deoxycytidine while cytidine preferentially labeled a nuclear pool of dCTP selectively drawn on for DNA synthesis. The very efficient utilization of both thymidine and deoxycytidine as DNA precursors suggests that some incorporation of free (i.e., unchanneled) deoxynucleotides does occur, since these deoxynucleosides are not converted to ribonucleotides in mammalian cells.

It is possible that several of the observations of unexpected kinetics described earlier in this section may result from channeling of DNA precursors in a multienzyme complex, as discussed above. For example, the apparent preferential incorporation of exogenous thymidine or deoxyuridine into DNA[86] could be the result of channeling through a multienzyme complex that included various kinases, ribonucleotide reductase, and DNA polymerase. Such channeling might also explain why some antimetabolites which inhibit synthesis of DNA precursors sometimes have an effect on DNA synthesis disproportionate to their effect on deoxynucleoside triphosphate pools.[93,94] If the major flow of deoxynucleosides into DNA proceeds via channeled intermediates, an inhibition of flux through the complex would have a greater effect on incorporation into DNA than would be expected on the basis of the observed decreases in the concentrations of free nucleoside triphosphates. Further studies on the extent of channeling in intact cells are clearly required and would have important implications for our understanding of drugs altering dNTP pools.

B. Compartmentation in Cyclic Nucleotides

The remarkably varied response of cells or tissues to agents known to activate adenylate cyclase have led several investigators to propose that compartments for cAMP and cGMP exist within cells. These compartments are proposed to serve to restrict the access of cAMP to only selected protein kinases rather than activating all intracellular kinases. The evidence for such compartmentation is indirect, usually consisting of data showing a dissociation of changes in cAMP concentrations and a particular hormone-induced effect. The evidence for compartmentation of cyclic nucleotides within cells has been recently summarized.[95,96]

Shimizu and colleagues have also proposed that cAMP is formed from a specific subcompartment of ATP in brain slices.[97,98] This group found that [^{14}C]adenine labeled the cAMP of brain slices to a much greater extent than expected from specific activity of the ATP pool. This study, although provocative, suffers from the drawbacks of all studies employing tissue slices: there may be a marked heterogeneity of metabolism within the slice due to different cell types or even location of particular cells within the slice.

IV. LIMITATIONS ON EVIDENCE FOR COMPARTMENTATION

The evidence for compartmentation of acid-soluble purine and pyrimidine nucleotides presented above suffers from a number of limitations, some of which have already been considered. Thus formidable technical difficulties remain in the separation of subcellular organelles in order to examine intracellular compartmentation, and high activities of enzymes of nucleotide metabolism at least potentially can produce rapid changes in nucleotide concentrations during separation. In particular, very rapid changes may be expected in nuclear pools of deoxyribonucleoside triphosphates, as it is estimated that the total cellular dGTP pool is replaced in only 30 sec in cells synthesizing DNA.[99] This rapid metabolism requires correspondingly rapid separation methods and termination of enzyme action. Finally, methods used to isolate organelles should not allow the loss of nucleotides through leakage; at present this is not always the case.

Additionally, further study is needed to provide clear-cut direct evidence for (or against) compartmentation (as in the case of nuclei) or of the extent of compartmentation in intact cells (for nucleotides tightly bound to proteins). In this regard a method for the rapid separation of nuclei with high yield of nucleotides would greatly aid further investigation of nuclear compartmentation.

A second type of limitation concerns those types of compartmentation which have been deduced on the basis of what we have called "indirect" evidence, and hence for which no cytological or molecular basis is known with certainty. Here, quite simply, more work needs to be done to establish the bases for the apparent anomalies in radioactive precursor metabolism that have so often been observed. In particular, the possibility of intranuclear or even intranucleolar compartmentation needs to be considered.

Another type of limitation applies to the cultured cells that have been the subject of many studies of nucleotide compartmentation. Thus the cells in an asynchronously growing culture are a mixture of cells in the various phases of the cell cycle, and there is abundant evidence that the several phases of the cell cycle are characterized by differences with respect to the size of nucleotide pools, the rate of pool turnover, and the activities of enzymes of nucleotide metabolism.[100-103] For example, although G_1 cells may incorporate [^{3}H]thymidine into dTTP pools, these pools do not contribute to the incorporation of label into DNA. Alternatively, if G_1 cells had a significant dTTP pool which was only very poorly labeled with [^{3}H]thymidine in comparison with S phase cells, the measured average specific activity of dTTP in the whole asynchronous culture would underestimate the specific activity of dTTP in the cells actually synthesizing DNA. Even the effect of antimetabolites on nucleotide pools has been shown to be greatly influenced by the phase of the cell cycle.[104]

In addition, it also appears that the extent of compartmentation may also vary widely among different types of cells. This point is illustrated most clearly by the report of Khym et al.,[71] who employed identical methods in attempts to detect compartmentation of UTP in six cell lines. Only two of the six lines appeared to have compartmentalized pools and even these two were markedly different in the rate of equilibration between the two UTP pools that appeared to be discernible. It seems at this time that the extent of compartmentation among cell types remains unclear.

A few studies of nucleotide compartmentation have been carried out using animal tissues rather than cultured cells (e.g., compartmentation of cyclic nucleotides, considered above, and a study of compartmentation of pyrimidine nucleotides in the kidney.[105] The contribution of different cell types in the tissues must be considered in these cases, as the turnover times and concentrations of nucleotide types may vary widely and thereby account for the apparent discordance of nucleotide-specific activities and rates of nucleic acid synthesis. Small subpopulations of cells such as the vascular epithelia may contribute to the measured nucleotide pools. Even the location of similar cells within the tissue may contribute to heterogeneity: hepatocytes in different locations (perivenous vs. periportal) differ in their metabolism of glutamine, for example.[106] Thus before invoking intracellular compartmentation to explain unexpected labeling kinetics in tissues, the heterogeneity of cells in the tissue must be considered.

Finally, compartmentation of nucleotides really needs to be considered in concert with the localization of the individual enzymes that utilize them as substrates, inhibitors, or allosteric effectors. However this topic is beyond the scope of the present review.

V. IMPLICATIONS OF STUDIES OF NUCLEOTIDE COMPARTMENTATION FOR STUDIES OF NUCLEOTIDE METABOLISM

That there is considerable evidence for the intracellular compartmentation of purine and pyrimidine nucleotides has been indicated above. However, it is necessary to ask further,

what does this mean for experimental studies of nucleotide metabolism? First, all of the qualifications regarding this evidence, discussed above, need to be taken into account.

Next, the importance of compartmentation will depend on (1) the size of the nucleotide pool directly involved in the process being studied, (2) the size of the pool(s) not directly involved, and (3) the rate of equilibration between them. For example, the size of the mitochondrial compartment is small in most cells, and the rate of equilibration (at least of adenine nucleotides) appears to be rapid. Thus studies of nonmitochondrial processes probably will not be much affected by this type of compartmentation, though it might be very important for studies of intramitochondrial processes. In contrast, platelet granules contain a large proportion of total adenine nucleotides, and the rate of equilibration is very slow; clearly this pool has to be taken into account in studies of adenine nucleotide metabolism in the nongranular portion of this cell. Because of the present state of studies of nucleotide compartmentation, however, the magnitude of these factors in most cases simply is not known.

At a more general level, however, the possibility of compartmentation has several implications for researchers measuring nucleotide pools and the specific activities of these pools as a means of estimating rates of nucleic acid synthesis. First, it seems unwise to attempt to predict changes in the rate of a metabolic process based only on measured changes in pool sizes, as the change measured may only reflect the average effect, whereas the alteration in nucleotide concentration at the site of the enzyme utilizing the nucleotide may be quite different. This might be particularly true in the case of multienzyme complexes with tight channeling. In this situation the pool within the complex is likely to be a small element of the total cellular pool, and more sensitive to changes in rate of synthesis or utilization by the enzymes within the complex; this compartmented pool would determine the overall rate of the process in the cell. These considerations dictate the use of measurements of metabolic flux in conjunction with measurement of nucleotide pools and enzyme properties.

It has also been recognized for some time that the incorporation of radioactive nucleosides into DNA and RNA can only serve to estimate the apparent rates of their synthesis because factors which affect dilution of label without affecting nucleic acid synthesis will lead to apparent changes in the rate of nucleic acid synthesis. The recent results of Nicander and Reichard[92] indicate that even when the specific activity of the deoxyribonucleoside triphosphate pools is considered, this method may give inconsistant estimates of DNA synthesis due to compartmentation of pools. Further work is necessary to delineate the circumstances under which incorporation of labeled nucleosides can be used to measure the rate of nucleic acid synthesis rather than to merely compare rates under different conditions.

REFERENCES

1. **Krebs, H. A.,** Regulation of the concentration of low molecular weight constituents in compartments, in *Microenvironments and Metabolic Compartmentation,* Srere, P. and Estrabrook, R., Eds., Academic Press, New York, 1978, 3.
2. **Sies, H., Ed.,** *Metabolic Compartmentation,* Academic Press, New York, 1982.
3. **Srere, P. A. and Estabrook, R. W., Eds.,** *Microenvironments and Metabolic Compartmentation,* Academic Press, New York, 1978.
4. **Akerboom, T., Bookelman, H., Zuurendonk, R., and Tager, J.,** Intramitochondrial and extramitochondrial concentrations of adenine nucleotides and inorganic phosphate in isolated hepatocytes from fasted rats, *Eur. J. Biochem.*, 84, 413, 1978.
5. **Keppler, D. and Holstege, A.,** Pyrimidine nucleotide metabolism and its compartmentation, in *Metabolic Compartmentation,* Sies, H., Ed., Academic Press, New York, 1982, 147.

6. **Veech, R. L.,** Regulation of coenzyme potential by near equilibrium reactions, in *Microenvironments and Metabolic Compartmentation,* Srere, P. and Estrabrook, R., Eds., Academic Press, New York, 1978, 17.
7. **Kunz, W. and Bohme, G.,** Studien über die kompartmentierung von ATP in Mitochondrien aus Rattenleber und Aszites-Tumor-zellen, *Acta Biol. Med. Ger.,* 14, 250, 1965.
8. **Schwenke, W., Soboll, S., Seitz, H. J., and Sies, H.,** Mitochondrial and cytosolic ATP/ADP ratios in rat liver in vivo, *Biochem. J.,* 200, 405, 1981.
9. **Wilson, D. F., Nelson, D., and Erecinska, M.,** Binding of the intramitochondrial ADP and its relationship to adenine nucleotide translocation, *FEBS Lett.,* 143, 228, 1982.
10. **Heldt, H. W., Klingenberg, M., and Milovancev, M.,** Differences between the ATP/ADP ratios in the mitochondrial matrix and in the extramitochondrial space, *Eur. J. Biochem.,* 30, 434, 1972.
11. **Kleineke, J., Duls, C., and Soling, H.,** Subcellular compartmentation of guanine nucleotides and functional relationships between the adenine and guanine nucleotide systems in isolated hepatocytes, *FEBS Lett.,* 107, 198, 1979.
12. **Lavoinne, A.,** Repartition of ATP, ADP, and PO_4 in isolated hepatocytes from fed and fasted rats, *Biochimie,* 65, 71, 1983.
13. **Siess, E. A. and Wieland, O. H.,** Phosphorylation states of cytosolic and mitochondiral adenine nucleotides and of pyruvate dehydrogenase in isolated rat liver cells, *Biochem. J.,* 156, 91, 1976.
14. **Soboll, S., Scholz, R., and Heldt, H. W.,** Subcellular metabolite concentrations. Dependence of mitochondrial and cytosolic ATP systems on the metabolic state of perfused rat liver, *Eur. J. Biochem.,* 87, 377, 1978.
15. **Soboll, S. and Bunger, R.,** Compartmentation of adenine nucleotides in the isolated guinea pig heart stimulated by noradrenaline, *Hoppe-Seylers Z. Physiol. Chem.,* 362, 125, 1981.
16. **Bunger, R., and Soboll, S.,** Subcellular distribution of cardiac adenylates as evaluated by a nonaqueous extraction method, in *Regulatory Functions of Adenosine,* Berne, R. M., Rall, T. W., and Rubio, R., Eds., Martinus Nijhoff, Boston, 1983, 504.
17. **Klingenberg, M., and Heldt, H. W.,** The ADP/ATP translocation in mitochondria and its role in intracellular compartmentation, in *Metabolic Compartmentation,* Sies, H., Ed., Academic Press, New York, 1982, 101.
18. **Hartung, K-J., Bohme, G., and Kunz, W.,** Involvement of intramitochondrial adenine nucleotides and inorganic phosphate in oxidative phosphorylation of extramitochondrially added adenosine-5′-diphosphate, *Biomed. Biochim. Acta,* 42, 15, 1983.
19. **Klingenberg, M.,** Mitrochondria metabolite transport, *FEBS Lett.,* 6, 45, 1970.
20. **Berk, A. J. and Clayton, D. A.,** A genetically distinct thymidine kinase in mammalian mitochondria, *J. Biol. Chem.,* 248, 2722, 1973.
21. **Bogenhagen, D. and Clayton, D. A.,** Thymidylate nucleotide supply for mitochondrial DNA synthesis in mouse L cells, *J. Biol. Chem.,* 251, 2938, 1976.
22. **Bestwick, R. K., Moffet, G. L., and Mathews, C. K.,** Selective expansion of mitochondrial nucleoside triphosphate pools in antimetabolite-treated HeLa cells, *J. Biol. Chem.,* 257, 9300, 1982.
23. **Parsons, P. and Simpson, M. V.,** Deoxyribonucleic acid biosynthesis in mitochondria, *J. Biol. Chem.,* 248, 1912, 1973.
24. **Soeiro, R. and Ehrenfeld, E.,** Cytoplasmic and nuclear pyrimidine ribonucleotide pools in HeLa cells, *J. Mol. Biol.,* 77, 177, 1973.
25. **Davis, R. H.,** Metabolite distribution in cells. Two carbamyl phosphate gradients and their sources can be discerned in Neurospora, *Science,* 178, 835, 1972.
26. **Crandall, D. E., Lovatt, C. J., and Tremblay, G. C.,** Regulation of pyrimidine biosynthesis by purine and pyrimidine nucleosides in slices of rat tissue, *Arch. Biochem. Biophys.,* 181, 194, 1978.
27. **Wendler, P. A., Blanding, J. H., and Tremblay, G. C.,** Interaction between the urea cycle and the orotate pathway: studies with isolated hepatocytes, *Arch. Biochem. Biophys.,* 224, 36, 1983.
28. **Hagen, P. B. and Toews, C. W.,** Unavailability of chromaffin granule adenosine triphosphate for metabolic reactions, *Biochim. Biophys. Acta,* 71, 201, 1963.
29. **Hillarp, N., Nilson, B., Hogberg, B., and Leo, A. B.,** Adenosine triphosphate in adrenal medulla of the cow, *Nature (London),* 176, 1032.
30. **Holmsen, H. and Weiss, H. J.,** Secretable storage pools in platelets, *Ann. Rev. Med.,* 30, 119, 1979.
31. **Reimers, H., Packham, M. A., Cazenave, J., and Mustard, J. F.,** Effect of reserpine on adenosine uptake and metabolism and subcellular transport of platelet adenosine triphosphate in washed rabbit platelets, *Biochem. Pharmacol.,* 26, 1657, 1977.
32. **Siebert, G.,** The limited contribution of the nuclear envelope to metabolic compartmentation, *Biochem. Trans.,* 6, 5, 1978.
33. **Ove, P., Takai, S., Umeda, T., and Lieberman, I.,** Adenosine triphosphate in liver after partial hepatectomy and acute stress, *J. Biol. Chem.,* 242, 4963, 1967.

34. **Stark, D., Hannover, R., and Siebert, G.,** Nucleotides and coenzymes in nuclei isolated from rat liver, *Hoppe-Seylers Z. Physiol. Chem.*, 359, 1203, 1978.
35. **Skoog, L. and Bjursell, G.,** Nuclear and cytoplasmic pools of deoxyribonucleoside triphosphates in Chinese hamster ovary cells, *J. Biol. Chem.*, 249, 6434, 1974.
36. **Behki, R. M. and Schneider, W. C.,** Intracellular distribution of deoxyribosidic compounds in normal and regenerating liver and in Novikoff hepatoma, *Biochim. Biophys. Acta*, 61, 663, 1962.
37. **Adams, R. L. P.,** Phosphorylation of tritiated thymidine by L929 mouse fibroblasts, *Exp. Cell Res.*, 56, 49, 1969.
38. **Adams, R. L., Berryman, S., and Thomson, A.,** Deoxyribonucleoside triphosphate pools in synchronized and drug inhibited L929 cells, *Biochim. Biophys. Acta*, 240, 455, 1971.
39. **Bestwick, R. K. and Mathews, C. K.,** Unusual compartmentation of precursors for nuclear and mitochondrial DNA in mouse L cells, *J. Biol. Chem.*, 257, 9305, 1982.
40. **Maccioni, R. B. and Seeds, N. W.,** Residual nucleotide and tubulins ability to polymerize with nucleotide analogs, *J. Biol. Chem.*, 257, 3334, 1982.
41. **Hasselbach, W.,** Die Bindung von Adenosindiphosphate, von Anorganischem Phosphat und von Erdalkalien an die Strukturproteine des Muskels, *Biochim. Biophys. Acta*, 25, 562, 1957.
42. **Till, U., Kohler, W., Ruschke, I., Kohler, A., and Losche, W.,** Compartmentation of orthophosphate and adenine nucleotides in human red cells, *Eur. J. Biochem.*, 35, 167, 1973.
43. **Hultin, H. O. and Richardson, S. H.,** The binding of phosphate, pyrophosphate, and nucleotides to the structural protein of beef heart mitochondria, *Arch. Biochem. Biophys.*, 105, 288, 1964.
44. **Seraydarian, K., Mommaerts, W. F. H. M., and Wallner, A.,** The amount and compartmentalization of adenosine diphosphate in muscle, *Biochim. Biophys. Acta*, 65, 443, 1962.
45. **Van der Meer, R., Akerboom, T. P. M., Groen, A. K., and Tager, J. M.,** Relationship between oxygen uptake of perfused rat liver cells and the cytosolic phosphorylation state calculated from indicator metabolites and a redetermined equilibrium content, *Eur. J. Biochem.*, 84, 421, 1978.
46. **Saks, V. A., Ventura-Clapier, R., Huchua, Z. A., Preobrazhensky, A. N., Jr., and Emelin, I. V.,** Creatine kinase in regulation of heart function and metabolism. I. Further evidence for compartmentation of adenine nucleotides in cardiac myofibrillar and sarcolemmal coupled ATPase creatine kinase systems, *Biochem. Biophys. Acta*, 803, 254, 1984.
47. **Gankema, H. S., Groen, A. K., Wanders, R. J. A., and Tager, J. M.,** Measurement of binding of adenine nucleotides and phosphate to cytosolic proteins in permeabilized rat-liver cells, *Eur. J. Biochem.*, 131, 447, 1983.
48. **Yushok, W. D.,** Control mechanisms of adenine nucleotide metabolism of ascites tumor cells, *J. Biol. Chem.*, 246, 1607, 1971.
49. **Lomax, C. A., Bagnara, A. S., and Henderson, J. F.,** Studies of the regulation of purine nucleotide catabolism, *Can. J. Biochem.*, 53, 231, 1975.
50. **Jones, M. E.,** Pyrimidine nucleotide biosynthesis in animals: genes, enzymes, and the regulation of UMP biosynthesis, *Ann. Rev. Biochem.*, 49, 153, 1980.
51. **Mally, M. I., Grayson, D. R., and Evans, D. R.,** Catalytic synergy in the multifunctional protein that initiates pyrimidine biosynthesis in syrian hamster cells, *J. Biol. Chem.*, 255, 11372, 1980.
52. **Christopherson, R. I. and Jones, M. E.,** The overall synthesis of L-5, 6-dihydroorotate by multienzymatic protein pyr 1-3 from hamster cells. Kinetic studies, substrate channeling, and the effects of inhibitors, *J. Biol. Chem.*, 255, 11381, 1980.
53. **Otsuki, T., Mori, M., and Tatibana, M.,** Studies on channeling of carbamyl-phosphate in the multienzyme complex that initiates pyrimidine biosynthesis in rat ascites hepatoma cells, *J. Biochem.*, 92, 1431, 1982.
54. **Traut, T. W.,** UMP synthase: the importance of quaternary structure in channeling intermediates, *Trends Biol. Sci.*, July, 1983.
55. **Traut, T. W. and Jones, M. E.,** Kinetic and conformational studies of the orotate phosphoribosyl transferase: orotidine-5′-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells, *J. Biol. Chem.*, 252, 8374, 1977.
56. **Progobpol, S., Gero, A. M., Lee, C. S., and O'Sullivan, W. J. R.,** Orotate phosphoribosyltransferase and orotidylate decarboxylase from *Crithidia luciliae:* subcellular location of the enzymes and a study of substrate channelling, *Arch. Biochem. Biophys.*, 230, 285, 1984.
57. **Prem veer Reddy, G., Singh, A., Stafford, M. E., and Mathews, C. K.,** Enzyme associations in T4 phage DNA precursor synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3152, 1977.
58. **Prem veer Reddy, G. and Pardee, A. B.,** Multienzyme complex for metabolic channeling in mammalian DNA replication, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3312, 1980.
59. **Noguchi, H., Prem veer Reddy, G., and Pardee, A. B.,** Rapid incorporation of label from ribonucleoside diphosphates into DNA by a cell-free high molecular weight fraction from animal cell nuclei, *Cell*, 32, 443, 1983.

60. **Wickremasinghe, R. G. and Hoffbrand, A. V.,** Inhibition by aphidicolin and dideoxythymidine triphosphate of a multienzyme complex of DNA synthesis from human cells, *FEBS Lett,* 159, 175, 1983.
61. **Prem veer Reddy, G. and Pardee, A. B.,** Coupled ribonucleoside diphosphate reduction, channeling, and incorporation into DNA of mammalian cells, *J. Biol. Chem.*, 257, 12526, 1982.
62. **Spyrou, G.and Reichard, P.,** Ribonucleotides are not channeled into DNA in permeabilized cells, *Biochem. Biophys. Res. Commun.*, 115, 1022, 1983.
63. **Taheri, M. R., Wickremasinghe, R. G., and Hoffbrand, A. V.,** Metabolism of thymidine nucleotides synthesized via the *de novo* mechanism in normal, megaloblastic, and methotrexate-treated human cells and in a lymphoblastoid line, *Biochem. J.*, 196, 225, 1981.
64. **Taheri, M. R., Wickremasinghe, R. G., and Hoffbrand, A. V.,** Functional compartmentation of DNA precursors in human leukaemoblastoid cell lines, *Br. J. Haematol.*, 52, 401, 1982.
65. **Wickremasinghe, R. G., Yaxley, J. C., and Hoffbrand, A. V.,** Gel filtration of a complex of DNA polymerase and DNA precursor-synthesizing enzymes from a human lymphoblastoid cell line, *Biochim. Biophys. Acta,* 740, 243, 1983.
66. **Plagemann, P. G. W.,** Nucleotide pools of Novikoff hepatoma cells growing in suspension culture. II. Independent nucleotide pools for nucleic acid synthesis, *J. Cell Physiol.*, 77, 241, 1970.
67. **Plagemann, P. G. W.,** Nucleotide pools of Novikoff hepatoma cells growing in suspension culture. III. Effects of nucleosides in medium on levels of nucleotides in separate pools for nuclear and cytoplasmic RNA synthesis, *J. Cell Biol.*, 52, 131, 1972.
68. **Piper, P. W.,** The incorporation of uridine label into the RNA of mouse embryo cells does not always reflect the labelling of the major cellular UTP pool, *FEBS Lett.*, 131, 373, 1981.
69. **Goody, H. E. and Ellem, K. A. O.,** Nutritional effects on precursor uptake and compartmentalization of intracellular pools in relation to RNA synthesis, *Biochim. Biophys. Acta,* 383, 30, 1975.
70. **Bolcsfoldi, G., Poels, L., and Eliasson, E.,** RNA metabolism in human cells during amino acid deprivation, *Biochim. Biophys. Acta,* 228, 664, 1971.
71. **Khym, J. S., Jones, M. H., Lee, W. H., Regan, J. D., and Volkin, E.,** On the question of compartmentalization of the nucleotide pool, *J. Biol. Chem.*, 253, 874, 1978.
72. **Falkenthal, S. and Lengyel, J. A.,** Measurement of ribonucleotide pool specific activities by an in vivo method: comparison with an in vitro method, *Biochemistry,* 20, 312, 1981.
73. **Wiegers, V., Kramer, G., Klapproth, K., Rehpenning, W., and Hilz, H.,** Determination of mRNA half-life in HeLa cultures by a poly(A)-independent direct analysis of specific radioactivity of mRNA, *Eur. J. Biochem.* 50, 557, 1975.
74. **Wiegers, U., Kramer, G., Klapproth, K., and Hilz, H.,** Separate pyrimidine nucleotide pools for messenger-RNA and ribosomal-RNA synthesis in HeLa S3 cells, *Eur. J. Biochem.*, 64, 535, 1976.
75. **Brandhorst, B. P. and Humphreys, T.,** Synthesis and decay rates of major classes of deoxyribonucleic acid-like ribonucleic acid in sea urchin embryos, *Biochemistry,* 10, 877, 1971.
76. **Emerson, C. P. and Humphreys, T.,** A simple and sensitive method for quantitative measurement of cellular RNA synthesis, *Anal. Biochem.*, 40, 254, 1971.
77. **Dinauer, M. C. and La Marca, M. J.,** The size of the guanosine triphosphate pool active in the synthesis of stable RNA by stage 6 oocytes of Xenopus Laevis, *Exp. Cell Res.*, 101, 122, 1976.
78. **Genchev, D.,** Free pyrimidine nucleotide pool of Ehrlich ascites tumor cells. Characteristics related to quantitative studies of RNA metabolism, *Biochem. J.*, 188, 75, 1980.
79. **Genchev, D. D., Kermekchiev, M. B., and Hakjiolov, A. A.,** Free pyrimidine nucleotide pool of Ehrlich ascites tumor cells. Compartmentation with respect to the synthesis of heterogeneous nuclear RNA and precursors to ribosomal RNA, *Biochem. J.*, 188, 85, 1980.
80. **Chen, J.-J. and Jones, M. E.,** Effect of 5-phosphoribosyl-1-pyro-phosphate on *de novo* pyrimidine biosynthesis in cultured Ehrlich ascites cells made permeable with dextron sulfate 500, *J. Biol. Chem.*, 254, 2697, 1979.
81. **Chen, J.-J. and Jones, M. E.,** Effect of 6-azauridine on *de novo* pyrimidine biosynthesis in cultured Ehrlich ascites tumor cells. Orotate inhibition of dihydroorotase and dihydroorotate dehydrogenase, *J. Biol. Chem.*, 254, 4908, 1979.
82. **Losman, M. J. and Harley, E. H.,** Evidence for compartmentation of uridine nucleotide pools in rat hepatoma cells, *Biochim. Biophys. Acta,* 521, 762, 1978.
83. **Puckett, L. and Darnell, J. E.,** Essential factors in the kinetic analysis of RNA synthesis in HeLa cells, *J. Cell Physiol.*, 90, 521, 1976.
84. **Dammgen, J. W. and Scholtissek, C.,** Cellular RNA and influenza-virion RNA are synthesized from different pools in chick embryo cells, *Eur. J. Biochem.*, 59, 51, 1975.
85. **Birch, B. and Turnock, G.,** Transfer RNA and ribosomal RNA are synthesized from the same pyrimidine nucleotide pool, *Biochem. J.*, 208, 89, 1982.
86. **Fridland, A.,** DNA precursors in eukaryotic cells, *Nature (London) New Biol.*, 243, 105, 1973.
87. **Mathews, C. K.,** Giant pools of DNA precursors in sea urchin eggs, *Exp. Cell. Res.*, 92, 47, 1975.

88. **Mathews, C. K. and Sinha, N. K.,** Are DNA precursors concentrated at replication sites?, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 302, 1982.
89. **Wittes, R. E. and Kidwell, W. R.,** A kinetic approach to the determination of the S phase pool size of thymidine triphosphate in exponentially growing mouse L cells, *J. Mol. Biol.*, 78, 473, 1973.
90. **Kuebbing, D. and Werner, R.,** A model for compartmentation of *de novo* and salvage thymidine nucleotide pools in mammalian cells, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3333, 1975.
91. **Baumunk, C. N. and Friedman, D. L.,** Absence of an effect of amethopterin and 5-fluorodeoxyuridine upon levels of thymidine triphosphate in HeLa cells, *Cancer Res.*, 31, 1930, 1971.
92. **Nicander, B.and Reichard, P.,** Dynamics of pyrimidine deoxynucleoside triphosphate pools in relationship to DNA synthesis in 3T6 mouse fibroblasts, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 1347, 1983.
93. **Lowe, J. K. and Grindey, G. B.,** Inhibition of growth rate and deoxynucleoside triphosphate concentrations in cultured leukemia L1210 cells, *Mol. Pharmacol.*, 12, 177, 1976.
94. **Grindey, G. B., Wang, M. C., and Kinahan, J. J.,** Thymidine induced perturbations in ribonucleoside and deoxyribonucleoside triphosphate pools in human leukemic CCRF-CEM cells, *Mol. Pharmacol.*, 16, 601, 1979.
95. **Haynes, J. S. and Brunton, L. L.,** Functional compartments in cyclic nucleotide action, *J. Cyclic Nucleotide Res.*, 8, 1, 1982.
96. **Earp, H. A. and Steiner, A. L.,** Compartmentalization of cyclic nucleotide-mediated hormone action, *Ann. Rev. Pharmacol. Toxicol.*, 18, 431, 1978.
97. **Shimizu, H., Creveling, C. R., and Daly, J.,** Stimulated formation of adenosine 3′, 5′-cyclic phosphate in cerebral cortex: synergism between electrical activity and biogenic amines, *Proc. Natl. Acad. Sci. U.S.A.*, 65, 1033, 1970.
98. **Shimizu, H. and Okayama, H.,** An ATP pool associated with adenyl cyclase of brain tissue, *J. Neurochem.*, 20, 1279, 1973.
99. **Reichard, P.,** From deoxynucleotides to DNA synthesis, *Fed. Proc.*, 37, 9, 1978.
100. **Skoog, K. L., Bjursell, K. G., and Nordenskjold, B. A.,** Cellular deoxyribonucleoside triphosphate pool levels and DNA synthesis, *Adv. Enzyme Regul.*, 12, 345, 1974.
101. **Hordern, J. and Henderson, J. F.,** Comparison of purine and pyrimidine metabolism in G_1 and S phases of HeLa and Chinese hamster ovary cells, *Can. J. Biochem.*, 60, 422, 1982.
102. **Cohen, A., Barankiewicz, J., Lederman, H. M., and Gelfand, E. W.,** Purine and pyrimidine metabolism in human T lymphocytes. Regulation of deoxyribonucleotide metabolism, *J. Biol. Chem.*, 258, 12334, 1983.
103. **Synder, R. D.,** Deoxyribonucleoside triphosphate pools in human diploid fibroblasts and their modulation by hydroxyurea and deoxynucleosides, *Biochem. Pharmacol.*, 33, 1515, 1984.
104. **Kyburz, S., Scaer, J., and Schindler, R.,** Effects of methotrexate on thymidine triphosphate levels in Chinese hamster ovary cells, *Biochem. Pharmacol.*, 28, 1885, 1979.
105. **Cortes, P., Levin, N. W., Dumler, F., Venkachalam, K. K., Verfhese, C. P., and Bernstein, J.,** Incorporation of exogenous precursors into uridine nucleotides and nucleic acid, *Biochem. J.*, 182, 677, 1979.
106. **Haussinger, D.,** Hepatocyte heterogeneity in glutamine and ammonia metabolism and the role of an intercellular glutamine cycle during ureogenesis in perfused rat livers, *Eur. J. Biochem.*, 133, 269, 1983.