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Journal of Fisheries & Aquatic Science - Special Issue

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Subcellular Phosphorus Kinetics for Lake Ontario

by

D.R.S. Lean[1] and R.L. Cuhel[2]

NWRI Contribution Series #86-145

December, 1986

[1]National Water Research Institute
Canada Centre for Inland Waters
P.O. Box 5050, Burlington, Ontario
L7R 4A6, Canada

[2]Rosenstiel School of Marine and
Atmospheric Sciences
Department of Biology & Living Resources
4600 Rickenbacker Causeway
Miami, Florida 33149 , U.S.A.

Subcellular Phosphorus Kinetics for Lake Ontario Plankton

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D.R.S. Lean

National Water Research Institute
Canada Centre for Inland Waters
P.O. Box 5050, Burlington, Ontario L7R 4A6, Canada

and

R.L. Cuhel

Rosenstiel School of Marine and Atmospheric Sciences
Department of Biology and Living Resources
4600 Rickenbacker Causeway
Miami, Florida 33149, U.S.A.

MANAGEMENT PERSPECTIVES

Radioactive phosphate has been used to show that during periods of high phosphate demand in Lake Ontario, all the soluble phosphate was taken up and replaced in a few minutes. The present investigation extends these observations to the subcellular level where the partitioning of recently assimilated phosphate into a low molecular weight fraction, phospholipids, ribonucleic acid (RNA), and polyphosphate and deoxyribonucleic acid (DNA) reflected the nutrient status of the plankton population. Furthermore, the rate of change in distribution of these fractions provided a measure of the rates of exchange between the subcellular compartments. The role of phosphate storage as polyphosphate was illustrated. Our results showed that the Lake Ontario plankton population was phosphorus deficient to some extent throughout the entire study period from April to November.

PERSPECTIVE-GESTION

On a utilisé du phosphate radioactif pour montrer que durant les périodes caractérisées par une forte demande dans le lac Ontario, tout le phosphate soluble en présence est capté et remplacé en quelques minutes. Dans la présente étude, on s'est livré à des observations au niveau infracellulaire. En effet, le fractionnement du phosphate fraîchement assimilé en une fraction de poids moléculaire léger , des phospholipides, de l'acide ribonucléique (ARN), des polyphosphates et de l'acide désoxyribonucléique (ADN) permet de déterminer l'état de la population planctonique sur le plan de l'apport de substances nutritives. De plus, le taux de changement de la distribution de ces fractions permet de déduire le rythme des échanges entre les compartiments infracellulaires. Par ailleurs, Le rôle du stockage du phosphate sous forme de polyphosphate a été mis en évidence. Les résultats de l'étude révèlent que la population planctonique du lac Ontario a été caractérisée par une pénurie plus ou moins grave de phosphate pendant toute la durée de l'étude, soit d'avril à novembre.

Lean, D.R.S. and R.L. Cuhel. 1987. Subcellular phosphorus kinetics for Lake Ontario plankton. Can. J. Fish. Aquat. Sci. 43:000-000.

The subcellular distribution of recently incorporated $^{32}PO_4$ was used to demonstrate that plankton in the surface waters of Lake Ontario were phosphorus deficient to some extent throughout the entire study period from April to October 1982. Most (ca. 80%) of the initial uptake of carrier-free radioactive phosphate was in the low molecular weight (LMW) fraction. Ribonucleic acid (RNA) was the other major fraction. Although uptake was often complete within 1 hour, changes in the subcellular distribution continued for 6 to 20 hours with about half the LMW^{32}P being transferred to RNA. Both a phospholipid fraction and a fraction extracted with hot trichloroacetic acid (HTCA) containing DNA, and high molecular weight polyphosphate increased to 4-15 % of the total isotope incorporated. In contrast to these experiments where net P uptake was zero, with added phosphate net influx continued for periods in excess of 30 hours but the subcellular distribution was constant within 2-4 hours. About 80 % of the initial uptake was as LMW^{32}P and a rapid net synthesis of low molecular weight polyphosphate was observed. These patterns were common for both small and large plankton size classes.

D.R.S., Lean et Cuhel, R.L. 1987. La dynamique du phosphore infracellulaire dans le plancton du lac Ontario. Journal canadien de sciences halieutiques et aquatiques. 43:000-0000.

La distribution infracellualire de $^{32}PO_4$ fraîchement assimilé a servi à démontrer que le plancton des eaux de surface du lac Ontario a été caractérisé par une pénurie plus ou moins forte de phosphore pendant toute la durée de l'étude soit d'avril à octobre 1982. La majeure partie (environ 80 p. 100) du phosphore radioactif sans entraîneur capté initialement se trouvait dans fraction de poids moléculaire léger. L'acide ribonucléique constituait l'autre fraction importante. Bien que la totalité du phosphate ait été captée en une heure dans bien des cas, les changements affectant la distribution infracellulaire se poursuivaient pour une période allant de 6 à 20 heures au cours de laquelle environ la moitié du ^{32}P de poids moléculaire léger était transféré à l'acide ribonucléique. La proportion de l'isotope radioactif présent dans la fraction de phospholipides et celle extraite à l'aide d'acide trichloroacétique chaud renfermant de l'ADN et des polyphosphates de poids moléculaire élevé a augmenté de 4 à 15 p. 100 dans les deux cas. Par contre, lorsque la captation de P était nulle, l'assimilation nette s'est prolongée au delà de 30 heures après l'ajout de phosphate tandis que la distribution infracellulaire s'est stabilisée au bout de 2 à 4 heures. Le phosphore capté initialement représentait une proportion de 80 p. 100 du ^{32}P de poids moléculaire léger; on a constaté que la synthèse nette de polyphosphate de poids moléculaire léger s'est déroulée rapidement. Ces tendances ont été observées pour les catégories de micro-organismes de grande de même que de petite taille.

INTRODUCTION

Annual phosphorus loading estimates have been used in whole lake models to predict average summer chlorophyll concentrations and hypolimnetic oxygen depletion rates (Vollenweider and Kerekes 1983) but short term kinetic experiments have identified rapid turnover of various soluble and particulate phosphorus compartments (Rigler 1973; Lean 1973, 1984; Taylor and Lean 1984; and others as reviewed by Cembella et al. 1984a,b). Such observations have led to P deficiency indices for predicting the duration and degree of P limitation in lakes (Lean and Pick 1981; Lean et al. 1987). The present investigation extends our knowledge of plankton phosphorus metabolism to the subcellular level under both ambient and phosphorus enriched conditions.

Under extreme P deficiency, turnover times for dissolved orthophosphate are very short (ca. 10 min.). Isotopic equilibration of the various particulate and dissolved compartments occurs with carrier-free radioactive phosphate without any net increase in particulate phosphate. Influx is equal to efflux as phosphorus is simply exchanged between the particulate and dissolved forms (Lean and White 1983). However, since P-deficient plankton can rapidly assimilate phosphate when it becomes available (Lean et al. 1987), experiments with added phosphate illustrate patterns for net uptake and storage.

Phosphorus is an integral component of a number of biological macromolecules, principally ribonucleic acid (RNA),

deoxyribonucleic acid (DNA), membrane lipids and can be stored as polyphosphates in microorganisms (Harold 1966; Healey 1973; Rhee 1980; Cembella et al. 1984a,b). Nucleotides such as adenosine triphosphate (ATP) and other high energy phosphorus compounds provide the energy for most biosynthetic processes (protein, RNA, DNA, and lipid synthesis; contractile processes; active transport of elements). ATP is generated via substrate-level or oxidative phosphorylation and photophosphorylation. Like other inorganic nutrients (e.g. SO_4, NO_3, NH_4, CO_2), PO_4 enters the planktonic food web through autotrophic and heterotrophic microorganisms. It is made available for macromolecular synthesis or energy metabolism by esterification to organic carriers (ADP, glucose, etc.). The high energy intermediates thus produced are used for all other P-utilizing reactions. Phosphate differs from the other inorganic nutrients in that it rarely undergoes biological reduction to form C-P bonds (Kittrege and Roberts 1969). Almost all P-containing compounds are phosphate esters.

Animals lack the ability to take up soluble phosphorus directly and they excrete excess phosphate (Rigler 1973, Taylor and Lean 1984) but the fraction excreted depends on the C:P ratio in the food. At C:P ratios in excess of 140, zooplankton may become P limited (Olsen et al. 1986). In the following investigation the influence of zooplankton is insignificant for little labelling occurs during the experimental period.

A detailed kinetic analysis of the phosphorus pathways in

even a single organism has been elusive (Cembella et al. 1984b). Consequently, an attempt to measure subcellular metabolism for an entire plankton population would seem fanciful. Nevertheless, the distribution of recently acquired radioactive phosphate provides an operational classification of the phosphorus nutritional state of the plankton community. In the following experiments, the criterion used for phosphorus deficiency was that phosphate additions to P-sufficient plankton populations should not alter the labelling patterns of subcellular distribution of recently incorporated $^{32}PO_4$. A shift to P-storage would suggest that the population was P deficient. Changes in subcellular distribution of radioactive phosphorus were monitored with and without added phosphate during the summer of 1982 in Lake Ontario.

MATERIALS AND METHODS

Samples were collected at stations 401 and 403 (Lean 1987; Simons and Schertzer 1987) in Lake Ontario and compared to samples from Jacks Lake, a smaller lake of similar trophic state on the edge of the Canadian Shield (Lean and White 1983). Carrier-free $^{32}PO_4$ with or without unlabeled PO_4 (25-35 ug $P.L^{-1}$) was added to steam-cleaned glass or polycarbonate bottles filled with water from 5 m depth. Final activities were about 40,000 (no added P) to 100,000 (with added P) $DPM.mL^{-1}$. Soon after isotope addition and at intervals thereafter, 10 mL subsamples were filtered through Whatman GF/F filters and rinsed with 5 mL

filtered lake water. The cell-retaining portion of the filter was excised using a punch funnel then frozen immediately (Cuhel et al. 1983).

Samples were treated by serial extraction to separate subcellular fractions. Filters were ground in cold 10 % trichloroacetic acid (TCA), centrifuged (5000 x g, 20 min), and the residue rinsed with 10 % TCA, yielding the low molecular weight (LMW) fraction which contains orthophosphate, pyrophosphate, short chain polyphosphates (PolyP a) and a wide variety of organic phosphate esters such as sugar phosphates, nucleotides, etc.

The residue was then extracted with 80 % EtOH (60 ^{o}C, 20 min), centrifuged, and re-extracted with 80 % EtOH:Diethyl ether (1:1, v:v; 60 ^{o}C, 20 min). After centrifugation, the combined supernatants constitute the lipid fraction. Occasionally, "lipid" fractions were further separated into alcohol-soluble, ether-insoluble (protein) and alcohol-soluble, ether-soluble (lipid) components. More than 90 % of the total label in the alcohol-soluble fraction was partitioned into the ether phase (lipid). Since ^{32}P was never significant in the ether-insoluble portion, this separation was not routinely performed.

After lipid extraction, the residue was dissolved in 1M KOH and heated at 60 ^{o}C for 1 h, after which the suspension was neutralized with 2 M HCl and made to 10 % TCA. Following 20 min in ice, the sample was centrifuged, and the pellet rinsed with 10

% TCA. The combined supernatants contain the cellular ribonucleic acid (RNA). No attempt was made to isolate polyphosphate fractions b and c during the KOH exctraction (Cembella et al. 1984b). The final step involved dissolving the residue in Ø.1 M NaOH and contains deoxyribonucleic acid (DNA), protein, and high molecular weight polyphosphate (PolyP d). Complications arising in the extraction of PolyP will be outlined in the discussion section.

Less than Ø.5 % of the total radioactivity resisted hot (9Ø - 1ØØ ^{o}C) acid hydrolysis (2 x 2Ø min) after the RNA extraction, indicating negligible ^{32}P labeling of protein. Whole fractions were counted in PCS (Amersham) for 2Ø min or 4Ø,ØØØ counts on a Searle Mark III liquid scintillation counter.

RESULTS

Seasonal patterns

Uptake of carrier-free $^{32}PO_4$ was generally slower during spring and fall compared to summer (Lean et al. 1987). Subcellular incorporation patterns for populations exhibiting slow phosphate turnover are exemplified by the experiment conducted at the mid-lake station (4Ø3) on 2Ø October (Fig. 1). Despite low soluble reactive phosphate (SRP) of 1 ug P. L^{-1} and warm water (12.2 ^{o}C) the turnover time was 12.9 hours. Fifty percent of the added carrier-free ^{32}P-PO_4 was filterable after 21 h, and when assayed with added phosphate (33 ug. L^{-1}) for the

same period, uptake was reduced to 6 %.

The distribution of the cellular ^{32}P as a percent of incorporated label provides additional information from that above (left panel, Fig. 2). The rapidity of phosphorus assimilation is emphasized by the fact that by the first sampling point (ca 10 sec), more that 20% of the ^{32}P had already passed through the LMW compartment into macromolecules. As accumulation of exogenous ^{32}P into LMW slowed, the LMW pool lost radioactivity hyperbolically with time primarily to RNA. By the end of the experiment, RNA was the principal subcellular fraction (40 %) while carrier-free isotope moved through LMW to RNA and to a lesser extent the HTCA and lipid fractions. With added P, LMW remained the principal form of assimilated phosphorus. The proportion in RNA, HTCA and lipid fractions was lower. After 21 hours, uptake was neither complete nor a steady state of the subcellular fractions achieved in either case.

A contrasting regime is depicted by an experiment on 9 June along the north shore (station 401) where the thermal bar was present and the temperature had risen to 10.8 ^{o}C even thought the temperature at the mid-lake station was only 3.1 ^{o}C. This experiment provides an example of rapid phosphate turnover time (12 min). In fact, this was the most extreme P deficiency observed at the northshore station over the entire study period (Lean et al. 1986). Uptake of carrier-free phosphate was complete in less than 1 hour, but the isotope flow among subcellular

compartments continued for more than 18 hours (right panel of Fig. 2). The LMW fraction dropped progressively to 25 % of the assimilated ^{32}P with a corresponding increase in RNA to 47 %, HTCA to 15 %, and lipid to 12 %. In contrast, uptake of isotope in the P-enriched sample continued for more than 18 hours but the subcellular fractions had reached a steady-state within about 2 hours. With added P, the LMW fraction also contained about 80 % of the initial assimilated ^{32}P but instead of declining, the LMW^{32}P remained near 75 %. RNA increased from initial values near 15 % to about 20 %. The HTCA and lipid fractions stabilized within 4 hours at 1.5 and 3 % respectively.

The 9 June data are replotted (Fig. 3) as in figure 1 but are shown on an expanded scale over the first hour. The most significant feature is that the carrier-free ^{32}P remains in LMW form. Since there was little additional uptake during the next 19 h, the long equilibration times of the RNA and LMW compartments provide information on the exchange rates between these compartments. With added phosphate (lower panels) LMW continues to represent the predominant form and net uptake of phosphate continues over the entire period. This represents a tremendous and rapid loading of P into labile LMW pools available for either storage (polyP) or biosynthesis as needed.

With added phosphate only 22% (i.e. 7 ug P. L^{-1}) of the isotope was taken up but this respresents a doubling of the original particulate P concentration. Since LMW was being

resupplied with radioactivity it continued to increase (i.e. ^{32}P uptake and assimilation was greater than the rate of incorporation of LMW into RNA). The increase in RNA was about 1/4 that for LMW, and explains the constant distribution of about 20% and 80% for these two forms (Fig. 2). Our technique does not distinguish DNA from high molecular weight polyphosphate (see discussion) so the increase in HTCA could be labelling of either of these forms. Low molecular weight polyphosphate is in the LMW fraction (see below).

Experiments conducted at Jacks Lake provided a higher degree of P deficiency, even though the levels of chlorophyll and phosphorus are similar to that in Lake Ontario. Turnover times are routinely less than 5 minutes (Lean et al. 1987). Incorporation of carrier-free ^{32}P into subcellular components followed a similar pattern to that for P-deficient populations in Lake Ontario. Furthermore, the response to added P was also similar qualitatively, but there was a much greater proportion of the assimilated ^{32}P in the LMW component. With a particulate P concentration of 7 ug P. L^{-1}, the uptake velocity with added phosphate of 10 ug P. $L^{-1}.h^{-1}$ over the first hour represents a biomass specific increase of greater than 1 h^{-1}. By 22 hours almost all the added 33 ug P.L^{-1} was taken up, more than quadrupling P biomass, yet, over 90 % of this amount remained in the LMW fraction.

Pulse chase experiments were conducted at different times

during experiments such as those described above by additions of PO_4 (1 uM) to half of the carrier-free subsample. For example, the plankton at station 401 on 24 August were so P deficient that with added P (1 uM) particulate P increased 3 fold in less than 30 hours and doubled in just 30 minutes (Fig. 4). The uptake of carrier-free isotope was complete within 0.3 h with LMW retaining about 80% of the initial label. Since the uptake of isotope was complete, this would have the effect of flooding the LMW compartment with cold phosphate. Instead of decreasing, LMW^{32}P remained constant (dashed line) indicating that LMW^{32}P was not exchanging with the medium. ^{32}P-RNA did not increase as much as in the control with no P chase, reflecting the lower specific activity of the LMW compartment. The lipid and HTCA fractions also failed to increase (not shown) as much as in the sample with no P chase. Perhaps equally significant, the recently acquired ^{32}P was not lost from RNA after the P chase. This suggests that a simple compartment model with interconnecting exchange reactions does not apply, or is insignificant compared to the net uptake and assimilation processes which occur when phosphate was added. Without the P-chase, LMW^{32}P decreased with a corresponding increase in RNA. When P was added soon after uptake was complete, transfer to RNA was blocked. When added after the subcellular fractions had equilibrated, subsequent changes were not found. Even at higher levels of added P (20 uM), radioactivity was not chased from the cells nor from the rapidly "exchanging"

compartments.

Data in Table 1 represent a wide range of rates for uptake and subcellular distribution. These differences could be caused by a combination of factors including temperature, biomass, different species, different plankton size distribution or changes in the degree of phosphorus deficiency. In the carrier-free experiments, 37.2 ± 9.2 % (n=10) was in RNA. Temperatures did not seem to influence the subcellular distribution but may have increased the time to reach equilibrium. With added phosphate, RNA was 19.4 ± 6.0 % (n=8). Low % RNA values were found at 403 on 25 August during the peak of chroococcoid cyanobacteria (Pick and Caron 1987). Since added phosphate reduced the % as RNA and increased the proportion in the LMW fraction, it would seem that a certain degree of P deficiency was present in all populations throughout the experimental period, but further refinement of LMW is required (see below).

P Uptake by different sizes of microorganisms

The uptake of carrier free ^{32}P appeared to stop at 60-70 % in experiments where the turnover time of phosphate was rapid (Fig. 3 and Table 1). At such times, uptake is principally by bacteria and picoplankton (Lean et al. 1987). GF/F filters have a nominal pore size of 0.7 um and retain most of the phytoplankton but not all the bacteria. With added phosphate, uptake shifts to larger sized plankton and GF/F filters retain nearly as much isotope as the 0.2 um Nuclepore (Table 2).

Since there was an observed shift in the size distribution of the organisms responsible for isotope uptake with added phosphate, changes in subcellular distribution could be due to either the size of the organisms responsible for uptake or the phosphate enrichment itself.

To determine the influence of cell size on isotope distribution and to further describe components of the LMW compartment, the phosphomolybdate complex of the normal SRP assay (Strickland and Parsons 1972) was extracted with diethyl ether, isolating phosphate from low molecular weight organic phosphorus (POP) and low molecular weight polyphosphate (PP). Two experiments were conducted in Lake Ontario near station 4Ø3 on 23 July and 18 September 1984 but the most extreme example of P deficiency was again found in Jacks Lake on 11 August. The subcellular distribution of such populations were compared to the same population with added phosphate sufficient to shift a portion of the uptake to the larger plankton size classes. These results were compared to plankton samples that were prefractionated (less than 5 um) prior to the phosphate addition and from postfiltration (filter fractionated after isotope labelling). As previously noted (Table 1) the % as RNA was much higher in the carrier-free experiments than for those with phosphate added (Table 3). Samples prefiltered through 5 um Nuclepore were similar to the whole lake water samples showing that differences in distribution were not due to the size of the

plankton responsible for the uptake of phosphate. The less than 5 um fraction without added P exhibited features suggesting that phosphate was released during the prefiltration procedure. The uptake was principally by this fraction (see below) in the WLW sample, but after prefiltration uptake was slower. The percent uptake was more like that with added P, again suggesting that some P was released during prefiltration. The fraction containing low molecular weight polyphosphate and particulate organic phosphate (PP+POP) increased with added phosphate.

Postfiltration results are compared to the prefiltration values at 1.5 and 2.5 hours of incubation (Table 4). The amount as cellular phosphate appears to be slightly less with corresponding increases in PP+POP when larger cells are included but the results are very similar in all examples. The differences observed in the distribution of label within the cells could not be due to shifts in the size distribution of plankton responsible for uptake. Values for turnover time of phosphate for the Lake Ontario September experiment and the Jacks Lake experiment are shown on Table 5. Approximately 69% and 58 % of carrier-free uptake was due to the Ø.2-1.Ø um fraction in the Jacks Lake and Lake Ontario examples, respectively. This shifted to only 3.5 and 5.9 % with added P. The greater than 12 um fraction was only 4.7 % of the carrier-free uptake for the Jacks Lake sample but increased to 44 % with added P. The greater than 12 um uptake for the Lake Ontario sample also increased from 1.1 to 22.4 % with

added P. The efficiency of retention of activity of GF/F filters followed a similar pattern as in Table 2 increasing from 64 to 96 percent of the uptake measured using Ø.2 um filters with added phosphate.

Results from 18 September are presented to illustrate the patterns of labelling of the subcellular fractions. Figure 5 shows the pattern for carrier-free uptake of isotope for WLW. Initial uptake was principally as PO_4 (recall LMW = PO_4 + PP+POP) with PP+POP increasing more slowly. RNA, lipid and HTCA follow. By 8 hours, the distribution (Table 3) was maintained with PP+POP equal to about 25 % of the total uptake.

With added phosphate (Ø.6 M) about 8 % of the added isotope was taken up in 2 hours (Fig. 6) but by 23 hours this had increased to 24 %. During the initial rapid uptake phase PP+POP increased from about 2Ø to 44 %. For the remaining period, all fractions increased at a proportional rate making the subcellular distribution appear constant. The prefiltered fraction less than 5 um shows a similar pattern (Fig. 7) to that for whole lake water except that the capacity to assimilate phosphate was less. This may have been due in part to the lower biomass, but smaller cells may not be as P-deficient as larger cells (Lean et al. 1987). Nevertheless, the labelling pattern is similar to that for WLW. This supports the view that differences observed in the distribution of isotope (Tables 1 and 3) were due to the deficiency of the cells rather than shifts from small to larger

plankton with added phosphate.

DISCUSSION

In Lake Ontario, the subcellular distribution of radioactive phosphate followed a consistent pattern from April to October, 1982. RNA^{32}P values were near 40 % in carrier-free experiments but declined to near 20 % with added phosphate. With added P a corresponding increase in LMW^{32}P resulted from storage of LMW polyphosphate. Since there was no net increase in cellular phosphate in the carrier-free experiments, ^{32}P was simply equilibrating with the metabolically active particulate fractions.

While subcellular transformations occurred rapidly, PO_4 dominated the subcellular distribution during the first few minutes. As cellular $^{32}PO_4$ decreased, RNA and the fraction which includes particulate organic phosphorus and low molecular weight polyphosphate (POP+PP) increased. In most cells, low molecular weight phosphate esters (POP) represent about 20 % of the total particulate phosphate (Bieleski 1973). This would imply that little LMW PolyP was present. Although uptake of carrier-free isotope was complete within 1-3 hours, subcellular distribution changed for periods of up to 16 hours until the near steady state distribution shown on figure 8 was reached.

With the addition of phosphate, isotope uptake represents net increase in cellular phosphorus. Here the rate was more rapid

over the initial 1-3 hour period as the subcellular distribution shifted from that shown of figure 8 to that on figure 9. No longer was exchange between the particulate and soluble phosphate pools significant. Although uptake continued for long periods (greater than 20 h), within a few hours, all fractions increased at the same rate. If we assume that POP remained as only 20 % of the total, then LMWPolyP increased until it represented 30 percent of the recently incorporated phosphate.

Independent estimates of polymeric P storage products were measured using the selective action of 253 nm UV light against organic P esters followed by acid hydrolysis for polyP. The incorporation of ^{32}P into LMW-Poly P or PolyP a as measured above was equivalent to values in total polyP from this method. This means that little PolyP exists in the RNA fraction.

Reviews of existing literature (Harold 1966; Kuhl 1974; Cembella et al. 1984b) suggest that "luxury" phosphate uptake is principally directed to high molecular weight polyphosphate. This includes PolyP b and c in our RNA fraction and PolyP d in the HTCA fraction. In our experiments, HTCA was not a predominant subcellular fraction in any of the experiments with or without added phosphate. Arguing against PolyP b and c being important was the fact that the "RNA" fraction was lower in the phosphate enriched samples. Instead LMW, representing phosphate, LMW organic phosphorus compounds, and LMW polyphosphate was the principal fractions (Table 1).

Beever and Burns (1980) considered that polyP with less than 20 Pi units would be soluble in TCA while those with greater than 20 Pi units would be insoluble in TCA, but noted Harold's (1966) contention that all forms of polyP should be acid-soluble regardless of chain length. Because polyphosphates are strong polyanions and bear negative charges even at acid pH, they combine with, and precipitate, positively charged macromolecules, such as proteins. The amounts of "soluble" and "insoluble" polyP will therefore depend also upon the availability of the basic receptor. Thus the formation of acid-insoluble polyP is probably an analytical artifact. In our experiments 10 mL of Lake Ontario water gave sufficient radioactivity for the subcellular fractionation. For chemical analysis, over 500 mL would be required. This would concentrate proteins (and possibly metals) that would contribute to the formation of "acid insoluble" polyP. PolyP fractions b and c may have been included in our RNA fraction but since values from UV oxidation and acid hydrolysis for total polyP were near our values for LMW PolyP the quantity of b and c must be low. As Cembella (1984b) points out, Hooper (1973) may have been correct in his assertion that binding of polyphosphate fractions to protein-RNA complexes represent an artifact of extraction. The first step in Kuhl's (1974) extraction involved an ether extraction for lipids. Without the cold TCA treatment many enzymes remain active. This may also have contributed to the discrepency.

The subcellular distributions noted above apply irrespective of the plankton size class responsible for the uptake. Uptake from soluble phosphate esters cannot be determined but added esters reduced the uptake of carrier-free phosphate and shifted the subcellular distribution toward that found in enriched samples. This reaffirms the potential of P-esters to supplement planktonic P requirements (Cembella et al. 1984b, Pick 1987) if present in useful quantities.

Our subcellular fractionation experiments showed that the plankton were always phosphorus deficient to some extent. Larger algae dominate during turbulent conditions such as that found in spring and fall. Their higher half-saturation constants prevents them from depleting the phosphate to the same extent (Lean et al. 1986). Consequently, rapid phosphate turnover times are found only in the stratified periods. Under conditions of balanced growth, the subcellular distribution of label would be the same for experiments both with and without added phosphate. This was never found during the entire period of investigation in Lake Ontario.

Acknowledgement: The authors thank Dr. B. Kent Burnison and an anonymous reviewer for their comments and suggestions.

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Table 1. Time (h) to reach uniform isotopic distribution between particulate and dissolved fractions (UPT) and subcellular fractions (SCF). Also shown with the steady state subcellular distribution are corresponding temperature and chlorophyll a values.

Date	Expt	Temp (^{o}C)	Chlor a ($ug.L^{-1}$)	Time UPT	Time SCF	^{32}P Distribution (%) LMW	LIPID	RNA	HTCA
Station 401									
7 Apr	(- P)	1.2	3.7	+20	+20	-58	3	+37	2
9 Jun	(- P)	10.8	3.0	1	+18.4	-25	12	+47	16
	(+ P)	10.8	3.0	+18.4	2	75	3	20	2
29 Jun	(- P)	8.2	4.1	2	15	51	8	36	5
	(+ P)	8.2	4.1	+20	2	80	3	15	2
24 Aug	(- P)	14.5	4.4	2	16	45	6.5	40	8.5
	(+ P)	14.5	4.4	+20	2	79	2	19	0.4
Station 403									
10 Jun	(- P)	3.1	2.5	+18	+18	-65	6	+28	0.4
15 Jul	(- P)	15.7	6.7	1	-	32	10	42	16
	(+ P)	15.7	6.7	-	-	71	2	26	1
12 Aug	(- P)	18.8	2.5	1	12	55	9	30	6
	(+ P)	18.8	2.5	+20	2	80	3	15	2
25 Aug	(- P)	16.6	4.6	1	10	79	2	18	1
	(+ P)	16.6	4.6	+20	3	87	1	12	-1
22 Sep	(- P)	16.5	6.6	2	20	40	10	42	8
	(+ P)	16.5	6.6	+20	2	76	4	18	2
20 Oct	(- P)	12.2	3.9	+21	+21	-32	+11	+42	+15
	(+ P)	12.2	3.9	+21	+21	-58	+6	+30	+6
Jacks Lake									
17 Aug	(- P)	22.0	4.6	-1	20	32	8	44	16
	(+ P)	22.0	4.6	+20	2	94	1	5	0.4

Table 2. Percent uptake of carrier-free radioactive phosphate with (+P) and without (-P) added phosphate by different sized Nuclepore filters compared with Whatman GF/F glass fibre filters. The incubation time (IT) refers to the time when the comparison was made. Also shown is the phosphate turnover time (PTT).

Date	PTT (h)	IT (h)	Percent Uptake GF/F	Ø.2	1.Ø	5.Ø	12
Station 401							
7 Apr (-P)	67	18	-	1.2	Ø.8	Ø.4	Ø.2
9 Jun (-P)	Ø.21	2.6	6Ø.5	84.7	28.3	6.5	5.5
(+P)	52	6.5	12.5	13.5	12.8	5.8	4.7
29 Jun (-P)	Ø.6	Ø.9	61.Ø	66.Ø	19.9	5.9	4.6
(+P)	26.1	1.2	4.8	4.6	3.8	2.3	1.7
24 Aug (-P)	Ø.1	1.Ø	54.5	93.8	35.8	3.1	Ø.6
(+P)	1Ø.9	1.2	2Ø.Ø	21.6	18.8	2.6	1.Ø
Station 403							
15 Jul (-P)	Ø.15	1.5	56.Ø	99	34.3	5.6	2.1
(+P)	12.9	2.Ø	1Ø.6	12.5	1Ø.6	4.2	1.4
12 Aug (-P)	Ø.2	1.3	64.2	93.4	58.4	5.2	1.7
(+P)	15.8	1.5	8.Ø	8.3	7.3	2.1	Ø.4
25 Aug (-P)	Ø.3	1.5	67.7	95.6	52.2	1.5	Ø.7
(+P)	23.3	1.6	6.6	7.4	6.2	1.1	Ø.3
22 Sep (-P)	Ø.7	1.1	54.5	67.7	21.8	4.6	2.9
(+P)	28.4	1.3	4.5	4.5	3.9	2.7	1.5
2Ø Oct (-P)	12.9	1.5	1Ø.Ø	13.3	5.5	2.3	1.8
(+P)	112	2.Ø	1.7	1.8	1.5	Ø.9	Ø.7
Jacks Lake							
17 Aug (-P)	Ø.Ø8	1.5	64.3	96.8	29.6	11.Ø	4.8
(+P)	4.Ø	Ø.75	27.6	43.3	31.3	2Ø.7	11.9

Table 3. Time (h) to reach uniform isotopic distribution for uptake (UPT) and subcellular fractions (SCF) for whole lake water (WLW) and through 5 um with ($^{P}_{+}$) and without ($^{P}_{-}$) added phosphate. At isotopic equilibration the isotopic distribution within the particulate fraction is provided. LMW was subdivided into cellular phosphate (PO_4) and polyphosphate plus low molecular weight particulate organic phosphate (PP+POP).

Date Expt.	Time		^{32}P Distribution (%)				
	UPT	SCF	PO_4	PP+POP	LIPID	RNA	HTCA
Lake Ontario							
23 Jul WLW (-P)	15	15	32	20	8	35	5
+P (0.2 uM)	+24	10	20	55	2	18	5
<5 um (-P)	15	10	15	48	4	30	3
+P (0.2 uM)	+24	+24	31	-45	2	+20	2
18 Sep WLW (-P)	1.5	8	25	25	9	35	+6
+P (0.6 uM)	+30	12	32	44	6	16	+2
<5 um (-P)	4	12	42	18	6	29	+5
+P (0.6 uM)	+30	12	27	45	6	20	+2
Jacks Lake							
11 Aug WLW (-P)	0.3	4	23	14	13	44	+6
+P (0.6 uM)	+23	2	31	52	3	14	0.3
<5 um (-P)	0.5	2	31	29	8	30	+2
+P (0.6 uM)	+23	2	45	38	3	13	0.6

Table 4. Subcellular distribution (%) of radioactive phosphate plus added phosphate (Ø.6 M) for experiments conducted on 18 September for prefiltered (PRE) and postfiltered (POST) samples.

	Time (h)	Filter Size (um)	Subcellular Distribution				
			PO_4	PP+POP	Lipid	RNA	HTCA
POST	1.5	1	47.2	26.5	5.4	18.9	2.Ø
POST	1.5	3	45.7	34.4	4.4	14.5	1.Ø
POST	1.5	5	44.1	4Ø.Ø	3.3	11.9	Ø.7
POST	1.5	12	44.2	38.5	4.3	12.3	Ø.6
WLW	1.5		5Ø.Ø	35.Ø	3.5	1Ø.9	Ø.6
PRE	1.5	5	39.5	39.Ø	4.5	16.Ø	1.Ø
POST	2.5	1	44.5	31.4	5.Ø	17.4	1.7
POST	2.5	3	44.3	37.5	4.2	13.Ø	Ø.9
POST	2.5	12	4Ø.7	45.9	3.2	9.6	Ø.6
WLW	2.5		43.4	4Ø.Ø	4.Ø	12.Ø	Ø.6
PRE	2.5	5	3Ø.8	45.5	4.5	18.Ø	1.2

Table 5. Percent uptake of carrier-free radioactive phosphate at ambient and phosphate enriched (+P) concentrations by different sized Nuclepore filters compared with Whatman GF/F glass fibre filters. The incubation time (IT) refers to the time when the comparison was made. Also shown is the phosphate turnover time (PTT). Values for isotope uptake are expressed as a percent of that retained on Ø.2 um Nuclepore filters.

Date	PTT (h)	IT (h)	Percent Uptake GF/F	Ø.2	1.Ø	5.Ø	12
Jacks Lake							
11 Aug	Ø.1	19	62	1ØØ	3Ø.7	8.5	4.7
+P	3.Ø	19	97	1ØØ	96.5	53.3	44.Ø
Lake Ontario							
18 Sep	Ø.75	2.5	66	1ØØ	41.9	5.5	1.1
+P	15.3	2.5	95	1ØØ	94.1	38.4	22.4

FIGURE HEADINGS

Figure 1. Isotope uptake expressed as a percent of total added radioactivity (o), in LMW (●), RNA (▽), lipid (♦) and HTCA (■) as a function of incubation time (h).

Figure 2. Uptake of radioactive phosphate (o) and subcellular distribution expressed as a percent in the particulate fraction for LMW (●), RNA (▽), lipid (♦) and HTCA (■) for samples collected at station 403 on 20 October 1982 (left) and station 401 on 9 June 1982 (right).

Figure 3. Symbols as in Fig. 1. Changes over the first hour are shown on an expanded scale on the left.

Figure 4. Symbols as in Fig. 3. After 0.5 hours the sample with carrier-free isotope was divided in half and the 1 uM phosphate added. Changes in this chase experiment are shown with a dashed line.

Figure 5. Isotope uptake expressed as a percent of total activity (o), in particulate phosphate (Δ), low molecular weight polyphosphate plus particulate organic phosphate (▲), RNA (▽), lipid (♦) and HTCA (■) as a function of incubation time (h). Experiments were conducted at station near 403 on 18 September, 1984 at ambient phosphate concentrations. Changes over the first hour are shown on an expanded scale on the left.

Figure 6. Headings as for figure 5 but at phosphate enriched (0.6 uM) concentrations.

Figure 7. As in figure 6 but for the less than 5 um fraction only.

Figure 8. Steady state box model representing exchange between soluble phosphate and cellular phosphate. Other subcellular fractions are shown in proportion to equilibrated isotope distribution.

Figure 9. Distribution of radioactive phosphorus in the particulate fraction during uptake under P enriched conditions. Areas are in proportion to isotopic distribution.

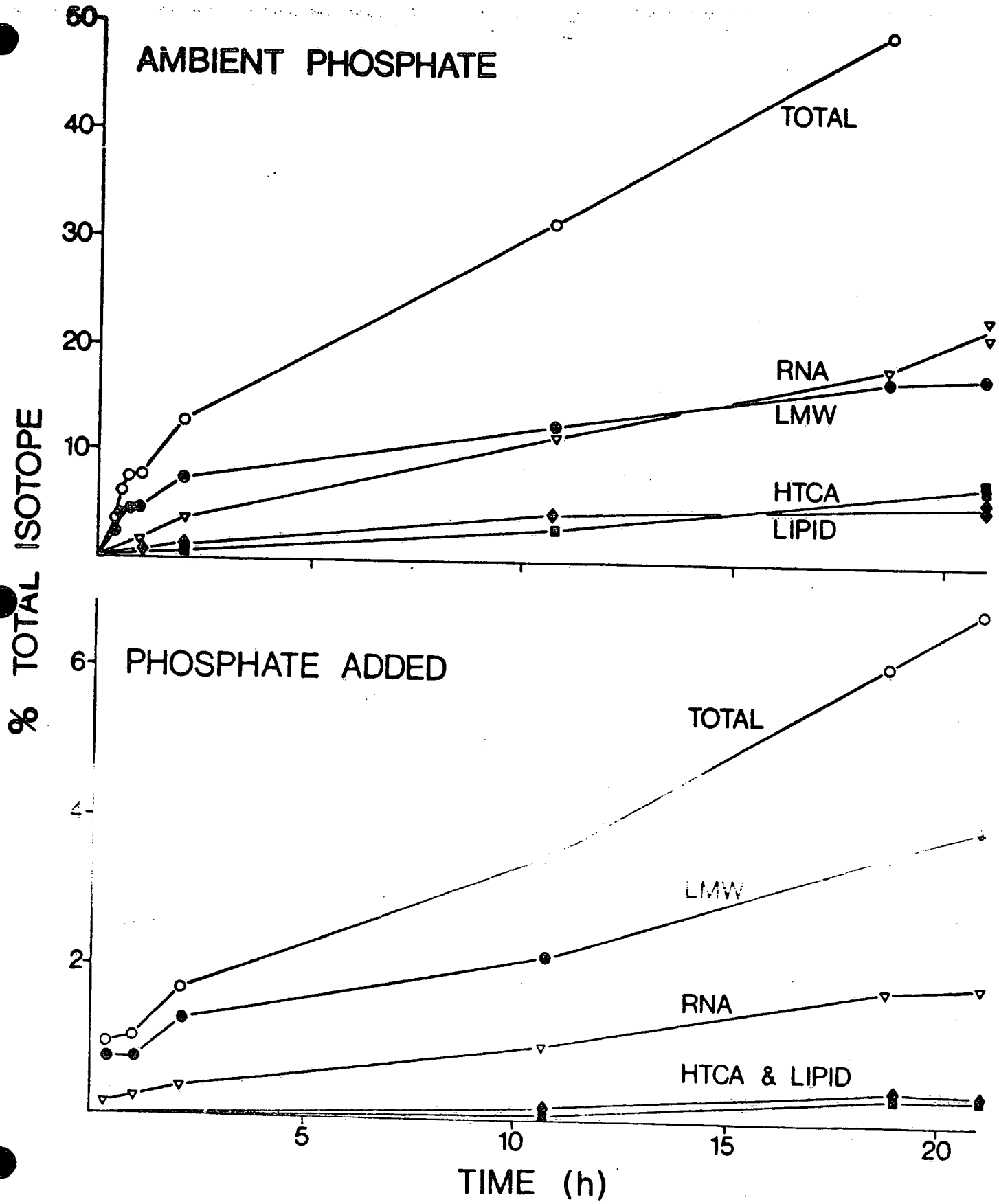


Fig. 1. Lean & Cuhel

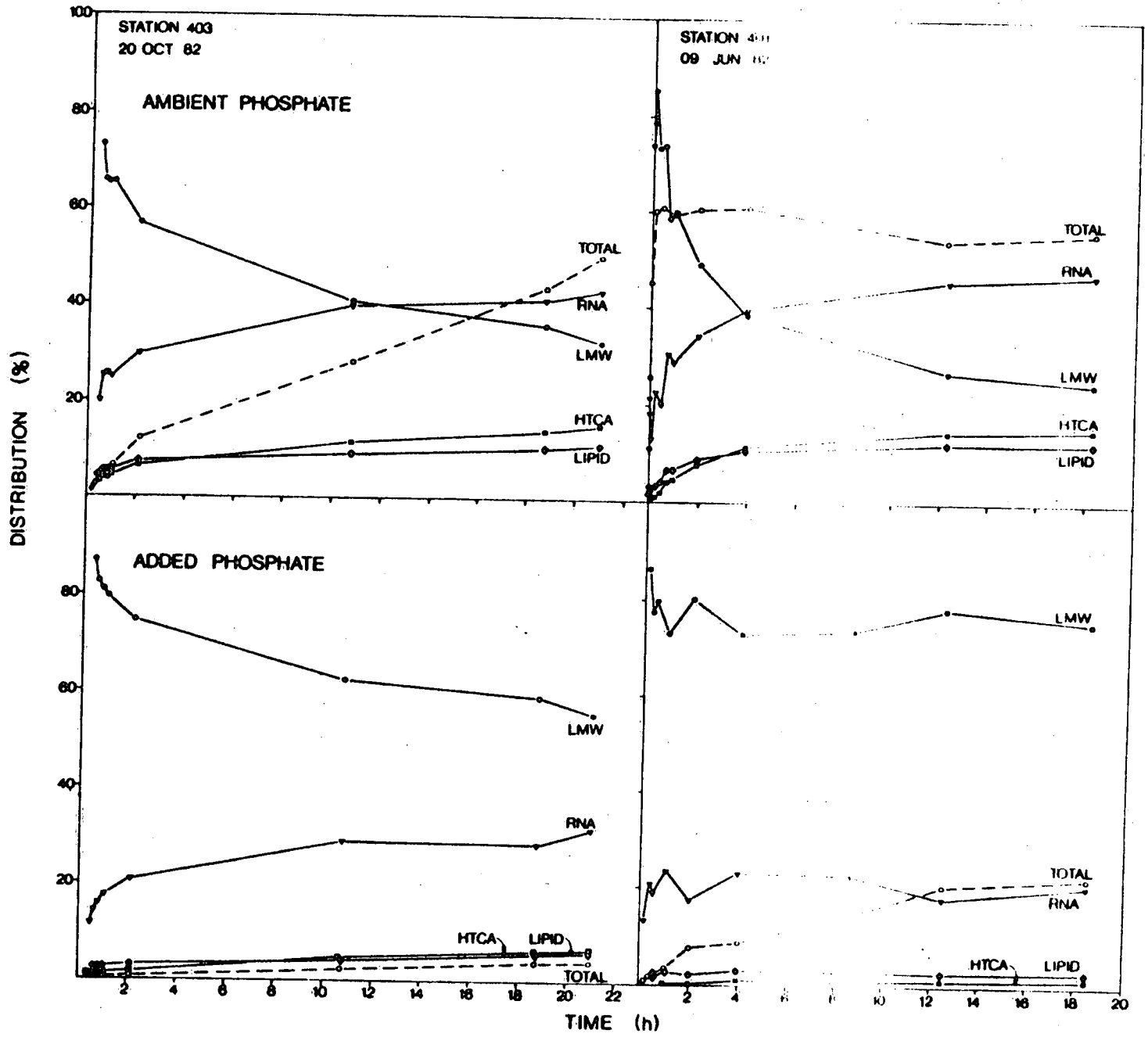


Fig. [illegible] Iturn & Cuhel

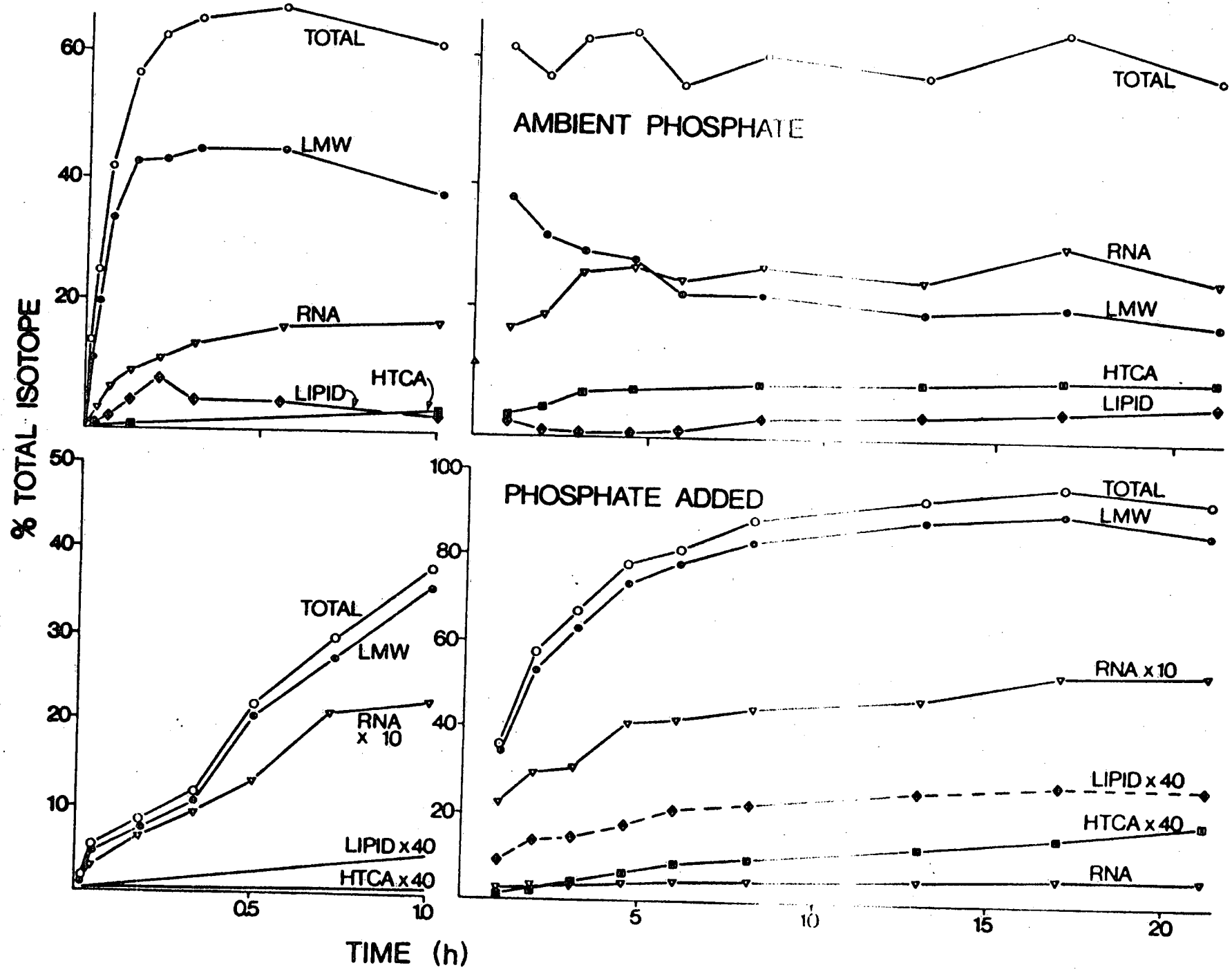

% TOTAL ISOTOPE
TOTAL
LMW
RNA
LIPID
HTCA
AMBIENT PHOSPHATE
PHOSPHATE ADDED
RNA x 10
LIPID x 40
HTCA x 40
TIME (h)

Fig. 3. [illegible]

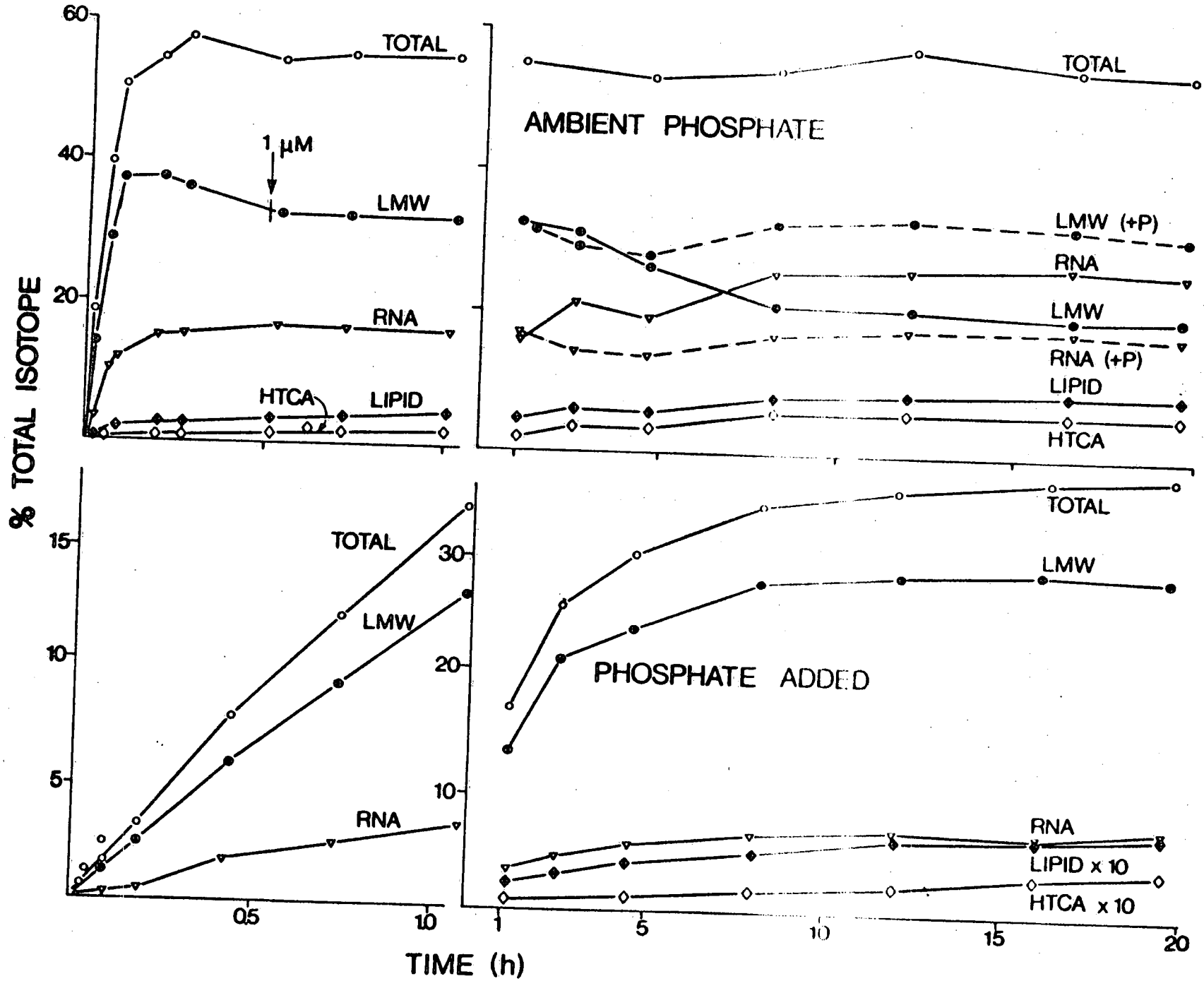


Fig. 4. Lean & Cuhel

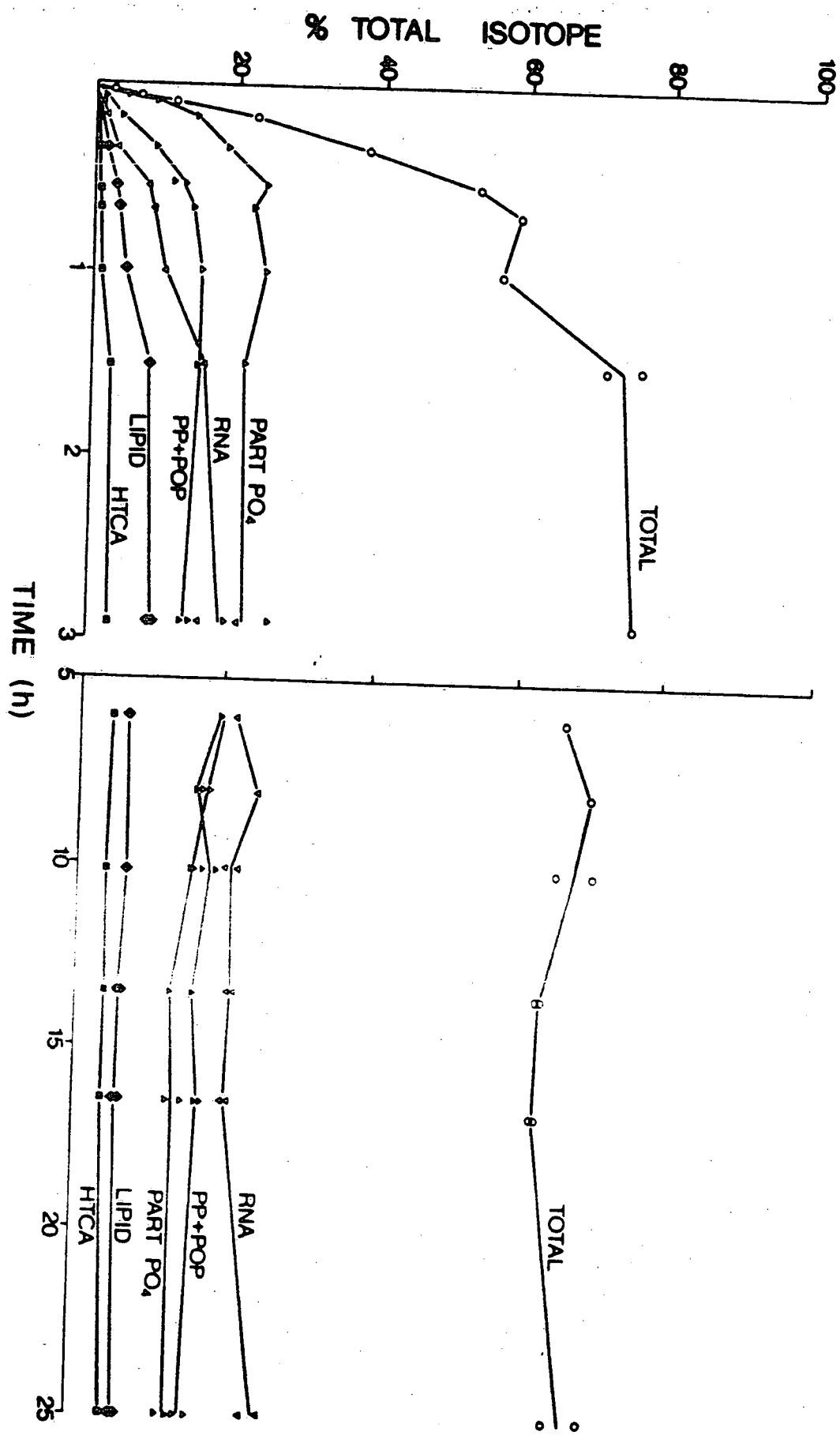


Fig. 5. Lean & Cuhel

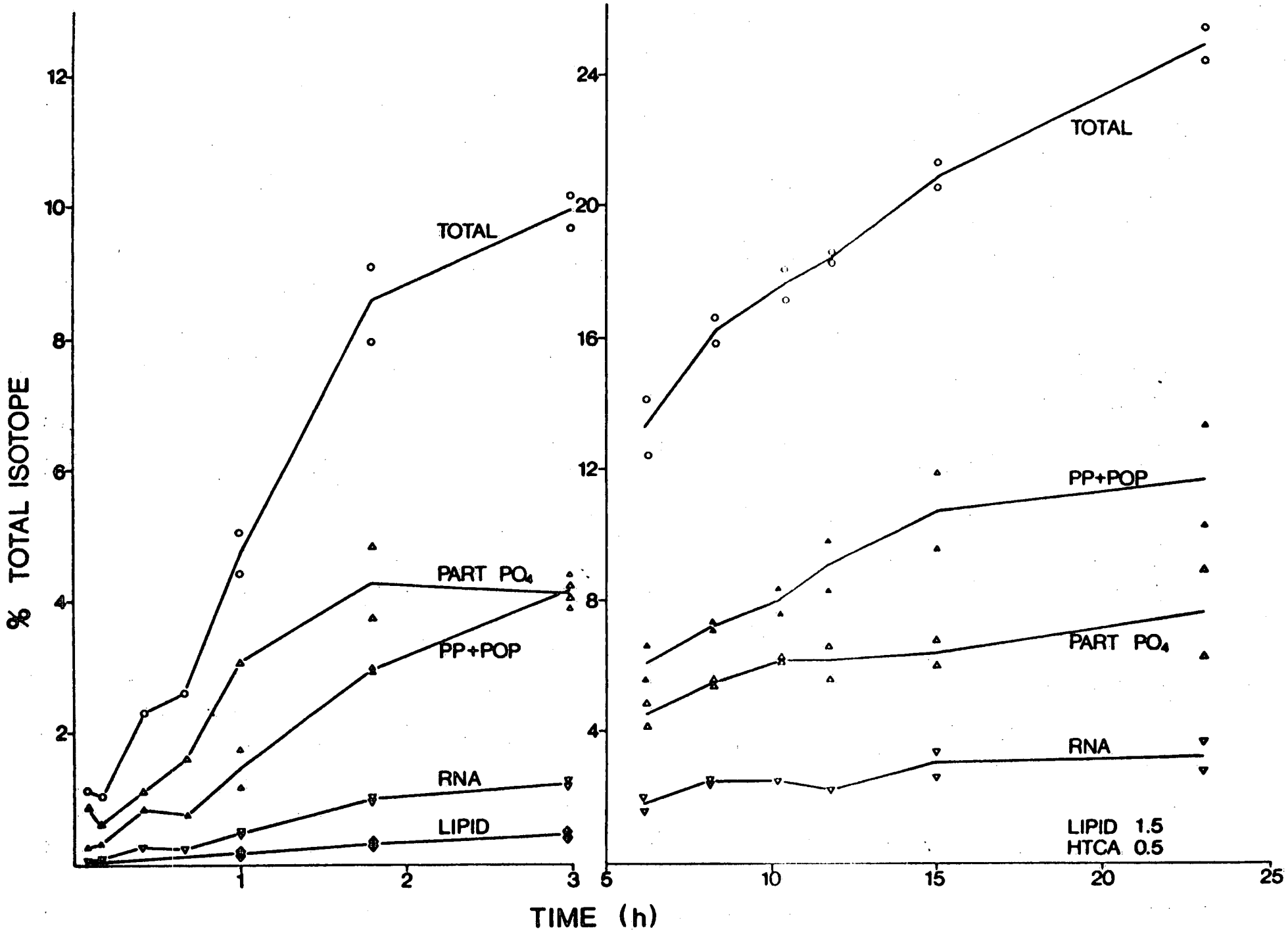


Fig. 6. Lean & Cuhel

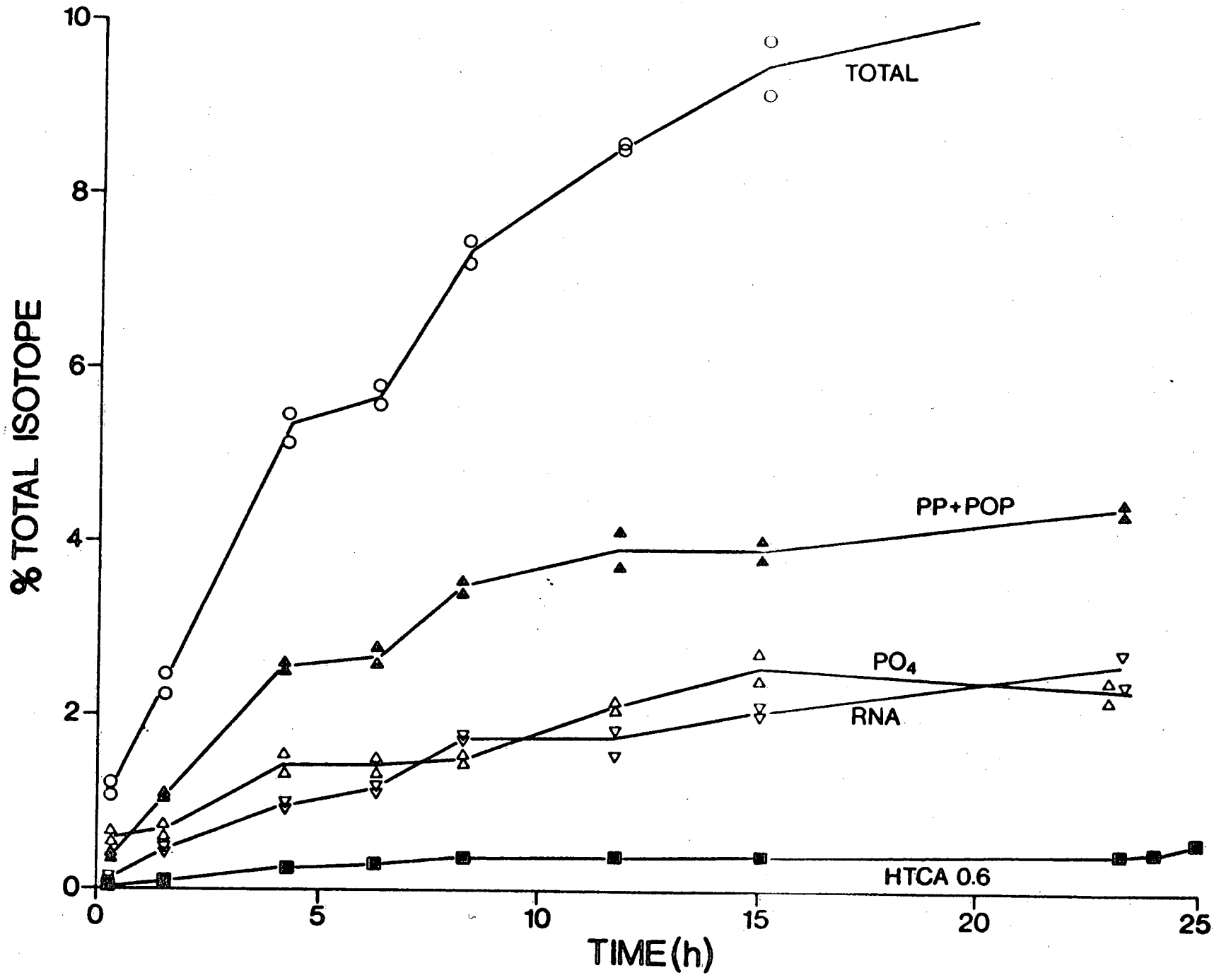
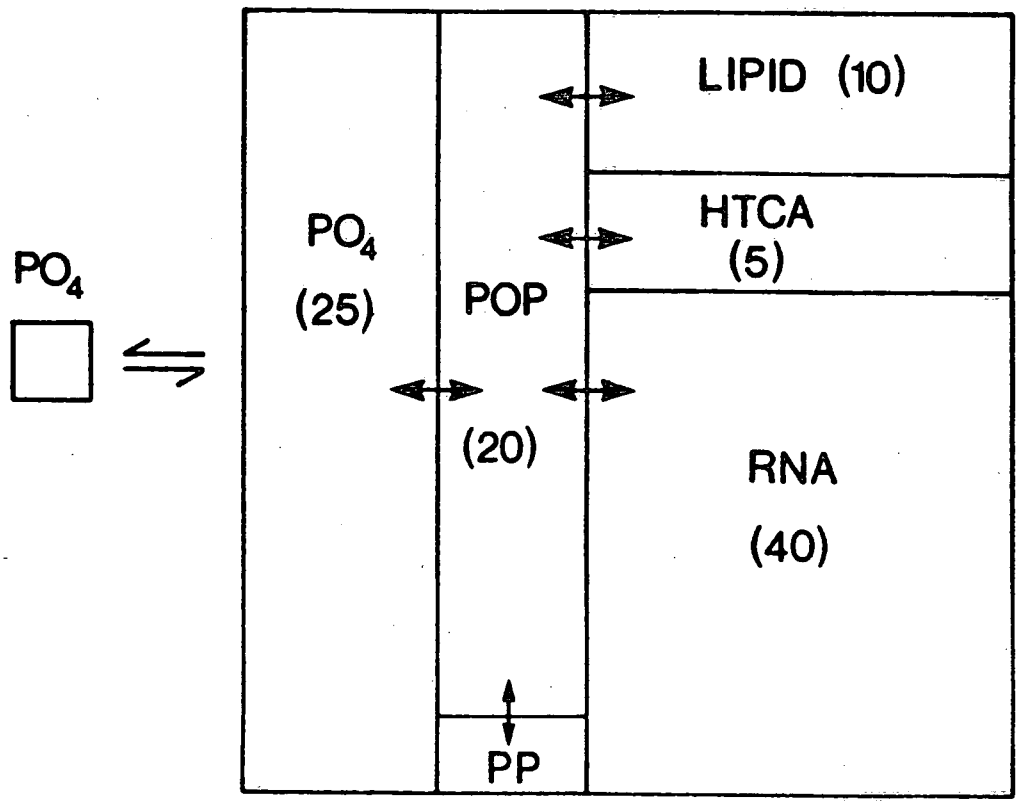


Fig. 7. Lean & Cuhe'


PO4
PO4
(25)
POP
(20)
LIPID (10)
HTCA
(5)
RNA
(40)
PP

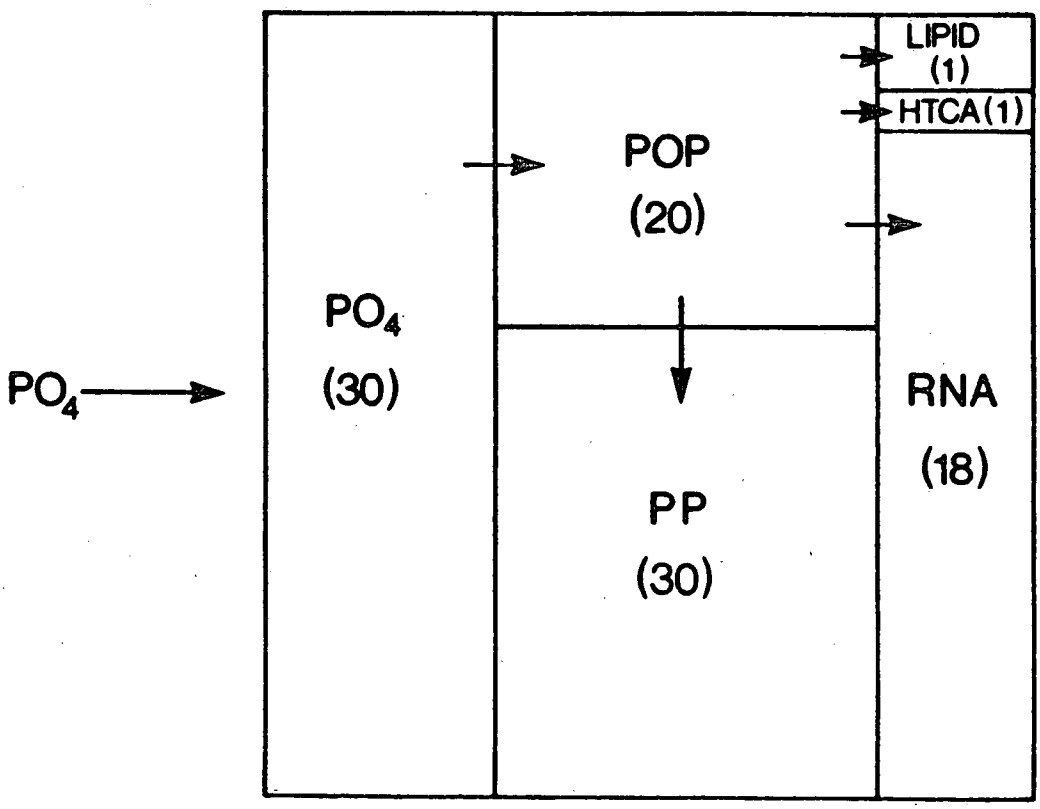


Fig. 9. Lean & Cuhel