We thank Miale *et al.* for their interesting comments regarding the use of erythrocyte morphology as a criterion of the carrier state in Duchenne muscular dystrophy. We agree with them that the approach has yet to be shown to have practical utility but we would also hold that a sample series consisting of three proposed carriers and two controls provides little basis for a decision.

The variability of their data as well as the generally lower percentages of distorted cells that they encounter suggest that there are differences of technique underlying the two sets of observations. Indeed, recent unpublished experiments in our laboratories have emphasized the importance of such factors as complete removal of plasma by thorough washing of the cells and the use of fresh glutaraldehyde in a fixing solution of proper final tonicity. In any event, we note that two of their three carriers and the majority of their Duchenne patients exhibited elevated percentages of distorted erythrocytes when compared with their controls. In other words, as far as their data go, they appear to be on the side of supporting our earlier observations.

Finally, with regard to erythrocytes from dystrophic patients, it should be mentioned that Appel *et al.* (*1*) have recently reported erythrocyte distortion from patients with Duchenne and myotonic muscular dystrophy. These investigators employ a preparation technique different from ours, omitting, for example, the saline washing, and the character of the distortion which they observe is likewise different, with dome-shaped cells in the dystrophic case.

DALE W. MATHESON
*Litton Bionetics, Inc., Kensington, Maryland 20795*
JOHN HOWLAND
*Committee on Biochemistry, Bowdoin College, Brunswick, Maine 04011*

**References**

1. S. H. Appel, A. D. Roses, C. G. Andrew, paper presented at the Third International Congress on Muscle Diseases, Newcastle upon Tyne, England (September 1974).

7 October 1974

## Phosphorus Dynamics in Lake Water: Contribution by Death and Decay

In his report Lean (*1*) appears to extrapolate from results of labeling studies of 1 to 24 hours in duration to conclusions concerning the entire dissolved organic phosphorus (DOP) pool in the natural water system. Although his work is interesting and seems to further elucidate the easily labeled and, thus, apparently the highly labile fraction of the DOP pool, several comments are in order.

First, his words "I identified the forms of $^{32}P$ in the filtrate . . ." are misleading since he has in fact not identified anything. He has *characterized* three fractions of labeled phosphorus, namely, the original orthophosphate, a high-molecular-weight fraction, and a low-molecular-weight fraction. Each of the latter two could quite reasonably include a host of compounds since Sephadex separations are based primarily upon molecular size differentiation.

Second, Lean proposes that the high-molecular-weight fraction is the result of a combination of the low-molecular-weight organic phosphorus with colloidal material in the lake water. In his model, then, he precludes the direct formation of high-molecular-weight phosphorus in the soluble or colloidal form. For the specific case where only 3 minutes of contact occurred between the organisms and the added $[^{32}P]PO_4$, it is reasonable to argue that the decay of organisms is not a likely source of the soluble organic phosphorus. However, if labeling of organic molecules within the organism can occur within 3 minutes, what evidence is there that release to the surrounding water has occurred by excretion instead of death and cellular lysis? Lean acknowledges that the experimentation took place at maximum biomass, a point where growth and death would be in balance.

On the other hand, if, in fact, not all the cellular organic phosphorus components received $^{32}P$ labeling in this short period of 3 minutes (that is, if some specific chemical compounds received no $^{32}P$ incorporation), then the model dynamics may indeed ignore a significant segment of the organic phosphorus pool. This nonlabeled segment could be comprised of an entirely different set of compounds from those represented by the "XP" and "Colloidal P" of Lean's model. However, these compounds could be released into solution predominantly by death and decay. Thus, Lean's repudiation of other authors' claims of release by death and decay (his references 12 and 13) based upon his model and sequence of experiments is not valid.

In fact, there is conclusive evidence (*2*), published prior to the final submission of Lean's report, that a significant fraction of the DOP in both laboratory algal cultures and natural waters is DNA or its fragments (7 to 10 percent of the DOP) capable of exclusion from Sephadex G-75 and G-100 gels. The DNA material represented roughly 50 percent of the total high-molecular-weight fraction. Three distinct responses were used to validate the identity of this isolated, high-molecular-weight material: (i) a deoxyribose-specific fluorescence analysis for DNA, (ii) enzymatic breakdown by deoxyribonuclease of the isolated high-molecular-weight peak, and (iii) conclusive isolation and identification of the bases adenine and guanine by two-dimensional, thin-layer chromatography (including cochromatographing standards and specific color reactions) after perchloric acid digestion of the isolated high-molecular-weight material.

That this material originated from the soluble compartment, independent of cellular damage during processing, was amply demonstrated (*2*) and in one case demonstration relied solely upon diffusive transport across a 0.22-$\mu$m membrane into sterile culture media. Although direct evidence to differentiate between direct excretion of DNA fragments by living organisms and release into solution by death and by subsequent decay was not sought, the presence of such fragments would certainly evoke temperance in denying the contribution of death and decay to the DOP pool.

Certainly, if Lean's experiments deal solely with excreted compounds originating from viable organisms, then most likely his results do not pertain to the entire DOP pool. In fact, he states, ". . . I concluded that no high-molecular-weight material was excreted, only XP."

Since the existence of DNA fragments in both algal cultures and several natural water systems has been clearly documented (*2*), these fragments must have been either excreted (contrary to Lean's hypothesis quoted above) or released by death and decay. Lean is

to be complimented on a significant and valid contribution to the understanding of phosphorus dynamics in natural waters. However, it should be emphasized that these results do not preclude a significant contributory role by death and decay in the overall DOP dynamics, as he implied.

ROGER A. MINEAR
*University of Tennessee, Knoxville 37916*

**References**

1. D. R. S. Lean, *Science* **179**, 678 (1973).
2. R. A. Minear, *Environ. Sci. Technol.* **6**, 431 (1972).

21 October 1973; revised 19 April 1974

In my model (*1*) I presented observations consistent with the kinetics of $[^{32}P]PO_4$ movement between the dissolved and particulate forms in lake water.

Death and decay are processes that are more important in conceptual models and in batch cultures than in lake communities. Minear's contention that at maximum biomass growth equals death is reminiscent of elementary explanations for the growth curves of batch cultures. I prefer to think of the biomass in a lake as being maintained through a balance between growth and zooplankton grazing, heterotrophic activity and sedimentation. Instead of a static biomass persisting throughout the summer months, a system is maintained in logarithmic phase and death rarely occurs. Grazing rates in eutrophic waters often exceed 80 to 100 percent each day; hence, death and decay are no more common in lakes than in chemostats with high turnover times.

Minear's criticism that I "characterized" the forms in the filtrate rather than "identifying" them is justified. The original draft of my report included comments on the existing techniques. The measurement of "soluble reactive phosphate" had been considered equivalent to orthophosphate, but it is now known to include artifacts which possibly include labile organic phosphorus as well. The "soluble unreactive forms," otherwise known as "dissolved organic phosphorus" (DOP), may be neither soluble nor dissolved but rather particles smaller than 0.45 $\mu$m (*2*). Furthermore, this fraction may not be organic. It is simply a substance that tests as phosphorus after perchloric acid digestion. By combining radioisotope tracer kinetics with gel filtration techniques [as stated in (*1*)], I identified the biologically important forms in a functional way. Space limitations did not permit such an extensive introduction in the final report.

One of the complications that has confused research on phosphorus in lakes is the lack of sensitive analytical techniques. By the time one has collected enough sample, cell damage has undoubtedly occurred and the products are difficult to distinguish from those produced through "death and decay."

Since 1970 when the original work was done which led to the model (*1*) in question, I have extended the experimental period from between 1 and 24 hours to 2 months in both hard water and soft water lakes. Some modifications in the existing model will need to be made, but the formation of so-called DOP seems to be the product of an excretion process and not the result of the "death" of the cell.

One should not get too upset over the lumping together of several compounds in a compartment I term "colloidal phosphorus" when I have committed an even greater "blunder" by lumping all living organisms, detrital material, clay particles, and other particulate materials under the heading "particulate phosphorus." My only excuse is that the model appears to be consistent with the observed kinetics.

The attention that Minear has given to DOP is certainly justified. Better identification will not only advance our knowledge of the role of phosphorus in lake water but may also provide some new insights into cellular metabolism.

D. R. S. LEAN
*Canada Centre for Inland Waters, 876 Lakeshore Road, Burlington, Ontario L7R 4A6*

**References**

1. D. R. S. Lean, *Science* **179**, 678 (1973).
2. F. H. Rigler, *Limnol. Oceanogr.* **13**, 7 (1968).

7 October 1974

## Primate Evolution: Were Traits Selected for Arboreal Locomotion or Visually Directed Predation?

In criticizing the proponents of the arboreal theory of the origin of primates, Cartmill (*1*) appears to overlook the principal reason why their theory was adopted, and if he did not make such an oversight, the differences between his view and theirs would be reduced. The arboreal theory was not postulated by Smith (*2*) and Le Gros Clark (*3*) to explain the replacement of tree shrew–like morphology with primate-like morphology, but rather to account for the emergence of tree shrew–like morphology from that of a terrestrial insectivore. Neither Smith nor Le Gros Clark believed that arboreal life per se accounted for primate differentiation, as is evinced by Smith's view (*2*, p. 39) that the elaboration of man from apes involved ". . . a continuation of those processes of evolution which we have been examining in the lowlier members of the Primate series." Thus, the proponents of the arboreal theory would readily agree with Cartmill's remark that tree dwelling, by itself, is not sufficient to transform an arboreal insectivore into a primate; some additional conditions, perhaps predation, as Cartmill suggests, is necessary for primate differentiation. In any case, both Cartmill and those he criticizes believed that increased reliance on vision was a key to understanding the evolution of primates.

It turns out that neither predation nor tree dwelling alone can provide a complete picture of changes in the visual nervous system in the various mammalian lines of descent. Cartmill uses the cat to illustrate the contention that predation results in the evolution of a highly developed visual system. In fact, the cat might be better used to show that predation does not result in a primate-like brain and visual apparatus. In presenting the cat as a highly visual animal, he overlooks the fact that not only is the acuity of the cat (*4*) and the dog much less than that of primates (*5*), but also, in psychological testing, visual stimuli have been shown to be much less compelling to carnivores than are auditory stimuli (*6*). It also should be noted that, irrespective of stereopsis, the visual anatomy of the cat is not very similar to that of the primate (*7*).

Finally, Cartmill argues that the case of the squirrel shows that an arboreal habitat does not produce primate traits. However, the brains of both the tree shrew and the squirrel do have many striking primate-like features; both possess enlarged occipital lobes and visual projections to the temporal