At various times oocytes were placed in water at 100° C. After several minutes, each was transferred to 0.5 ml of 1% sodium dodecyl sulphate plus 5 mM dithiothreitol (*p*H 8.0) and incubated at 50° C until dissolved. Samples of dissolved oocytes were placed on 23-mm diameter disks of Whatman No. 42 paper, air-dried and treated with cold and hot trichloroacetic acid (containing 1 mM NaH_2PO_2) and alcohol and ether to remove low-molecular-weight material, nucleic acid and lipid[2]. The disks were counted for ^{32}P-protein in a Beckman Lobeta II planchet counter and then used to determine total protein[6]. Figure 1*a* shows that, after a lag of several hours, the specific activity of the progesterone-treated oocytes increased with time, whereas that of the untreated oocytes did not.

After 16 h, 45 oocytes from each group were homogenised in strong salt solution[2], dialysed and chromatographed on a 23/0.9 cm TEAE-cellulose column[7]. Under these conditions lipovitellin and phosvitin eluted at positions 0.42 and 0.72, respectively. Samples from each fraction were assayed for ^{32}P-protein[3] (Fig. 1*b*). Neither lipovitellin nor phosvitin appeared to be specifically labelled in either case, and the increased amount of labelled material in progesterone-treated oocytes was found (*a*) initially percolating through the column, (*b*) between the lipovitellin and phosvitin peaks, and (*c*) eluting with the wash solution at the end of the run.

We conclude that there is an increase (although not intense) in protein phosphorylation during oocyte maturation, but that phosvitin is not involved. Phosvitin could serve as an energy source during embryogenesis, as suggested[1,8]. The dephosphorylation observed by Morrill and Murphy during early embryogenesis, however, probably involves some other protein(s). We do not yet know whether the increased phosphorylation during oocyte maturation results from an "activation of a protein kinase (possibly *via* cyclic AMP)." We have previously described a protein kinase from amphibian oocytes and eggs that is stimulated by cyclic AMP when histone is used as the substrate but not when phosvitin is the substrate[9].

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DR MORRILL REPLIES—Dr Wallace appears to confirm our observation[1] that release of the prophase block in amphibian eggs is associated with increased protein phosphorylation. The *in vitro* studies of Wallace and our *in vivo* studies are not, however, directly comparable. We injected ^{32}Pi directly into gravid females together with inducing levels of pituitary extract. It is our experience that isolated body cavity (ovulated) eggs take up and incorporate ^{32}Pi and would probably do so in both the body cavity and oviduct. The *in vivo* maturing eggs were thus exposed to ^{32}Pi for at least 24 h until released from the oviduct. In contrast, Wallace preincubated isolated oocytes with ^{32}Pi for 2 h and then transferred the oocytes to medium containing progesterone and unlabelled Pi. We have found that intracellular ^{32}Pi of excised oocytes exchanges rapidly with extracellular Pi. Thus, during the progesterone stimulus ^{32}P incorporation into protein would be limited to diminishing endogeneous supplies of phosphorylated nucleotide. This might contribute to the lower overall protein phosphorylation reported in the *in vitro* system, and possibly to differences in the protein species phosphorylated.

The main difference between our results and those of Wallace is the identification of the phosphorylated protein species. A comparison of a number of phosvitin extraction methods indicated that the major ^{32}P-labelled protein(s) of the *in vivo* ovulated frog egg could be isolated by the method of Mano and Lipmann[2]. This ovulated egg 'phosvitin' co-electrophoresed with commercial hen's (ovulated, unfertilised) egg phosvitin on SDS polyacryalamide gels. This 'phosvitin' differs from that characterised by Wallace[3] in that: (1) it has a lower phosphate content; (2) it is extractable with lower salt concentrations; and (3) it is associated with the cortical pigment granule fraction and not with the yolk platelets. This suggests that amphibian egg phosvitin exists more than one metabolically active form, one principal difference being the level of phosphorylation. In this regard, Mano and Lipmann[2] have shown that fish egg phosvitin can be separated into discrete peaks containing 3.2, 5.7, 7.2, and 9.5% P. The existence of discrete phosphorylation levels of the same protein backbone structure is particularly interesting.

Neither our maturing oocytes nor Wallace's were compared with the ideal control, that is oocytes completely free of follicle cells. In contrast to the ovulated eggs used in our experiments, oocytes dissected manually as described by Wallace retain a surface layer of intact follicle cells and these cells may also actively phosphorylate protein. When we injected ^{32}Pi into gravid females with no hormone stimulation we recovered ^{32}P-labelled protein in excised ovaries and manually dissected oocytes after 24–48 h *in vivo*. But, there was no significant ^{32}P incorporation into the 'phosvitin' fraction isolated as described above. This latter information was accidentally omitted from our final reduced manuscript, through the oversight of the authors.

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Tin mineralisation

SILLITOE's recent letter[1] on tin mineralisation and mantle hotspots is grossly misinformed about the age of the tin mineralisation in South West Africa. The tin deposits do occur in pegmatites but they are unrelated to the possible plume generated Jurassic–Cretaceous alkaline complexes in Damaraland. Instead the pegmatites are related to the Late Precambrian Damara orogen. Radiometric age determinations for a number of pegmatites are in the range 450 to 650 Myr (ref. 2.) I know of no tin mineralisation associated with the Mesozoic alkaline granites in South West Africa and they cannot, therefore, be used to support the contention of a relationship between tin-bearing silicic rocks and mantle hot spots.

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Primary structure of *E. coli* dihydrofolate reductase

IN a recent paper[1], the primary structure of *Escherichia coli* dihydrofolate reductase was reported from this laboratory. The paper also suggested a