matters arising

30 S DNA: intermediate or artefact?

SIR,—Goldstein and Rutman[1] have pulse-labelled the DNA of Ehrlich ascites cells growing intraperitoneally in a mouse with either ^{3}H or ^{14}C-thymidine. The DNA was subsequently extracted from the cells and its molecular weight measured on gradients of alkaline sucrose.

After short pulse times (< 2 min), the labelled DNA had a sedimentation coefficient of 9S indicating the presence of short intermediates in DNA replication ("Okazaki" fragments[2]). After longer times (10–30 min) they observed labelled DNA which sedimented somewhat more slowly than the bulk DNA (30S as opposed to 44S) and they claimed that this represented a discrete intermediate in the replication of DNA. This interpretation is improbable.

If a long molecule is labelled at one end (as happens with a short pulse label) and then sheared, the labelled molecules will always appear to have lower molecular weights than the bulk material. This artefact is a result of the different labelling patterns of pieces that derive from molecules labelled only at the ends, as compared with molecules labelled throughout their lengths. This effect has been described qualitatively in ref. 3 and a complete mathematical analysis can be found in ref. 4.

The observation of apparently slightly smaller lengths of pulse-labelled DNA (for example, 30S as opposed to 44S) is therefore not in itself evidence for a special class of DNA molecules. It merely demonstrates that the label goes on to the ends of growing DNA strands. This criticism does not apply to the observation of 9S DNA. The most plausible interpretation of the data is that there is an intermediate in DNA replication of about 0.4×10^{6} daltons and that this is linked directly on to the end of the growing DNA molecule.

Yours faithfully,

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Drs Goldstein and Rutman respond: The points from our study[1] of 'self-chase' Ehrlich ascites tumours which supported the DNA replication model involving sequential tandem ligations of 9S→30S and 30S→44S were: (1) maximal radioactive pulse-labelling of new 9S DNA by 2 min; (2) sharp peak transitions in alkaline sucrose gradients of labelled DNAs from alkaline-lysed cells, with no broad envelope of DNA sizes observed (2–120 min); (3) time of appearance of bulk-size labelled 44S DNA, 20–30 min, was best explained by ligation of 20 of the 9S chains to form 30S DNA and further ligation of 3 of 30S to form 44S; if 9S were directly linked to the end of a growing, long molcecule, as proposed by Lehmann and Ormerod[3,4], it should have taken 120 min to form 44S with an initial polymerisation rate of 600 deoxynucleotides per min; (4) at that rate of synthesis, excess DNA per cell would have been made in a 10 h S phase[1].

If ligation processes are under the control of replication fork(s)[1], before any ligation could begin, RNA primer molecules, to which Ehrlich cell Okazaki fragments are probably covalently linked[5,6], would have to be removed and replaced by DNA, as described for *E. coli*[7]. These latter mechanistic requirements with respect to 9S DNA taken in conjunction with our approximate kinetic analyses of 44S formation indicate that, like 9S, 30S DNA is a discrete replicative intermediate and not an artefact. The crux of this discussion rests on our choice between alternative interpretations of the data.

We have subsequently shown that bifunctional nitrogen mustard interferes with both synthesis of 9S DNA chains and their eventual step-wise conversion to 44S through 30S DNA[8].

Yours faithfully,

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Impact erosion by jets of dilute polymer solutions

SIR—Results described in the letter by Kudin *et al.*[1] increase the number of types of materials which have been cut by an impacting jet containing traces of soluble polymers. The hypothesis propounded by the authors, that increase in erosion capability is due to the rigidity of large aggregates of the polymer when subjected to high loading rates is not, however, correct.

In work carried out at the Universities of Leeds and Missouri-Rolla[2,3] work on polymer addition has shown an improvement over plain water jets in impact erosion of rock (Fig. 1). The fluid was filtered at 10 μm before entering the pumping system. This would hold back any large aggregates which Kudin *et al.*, suggested act as solid particles at short time scales and cause the

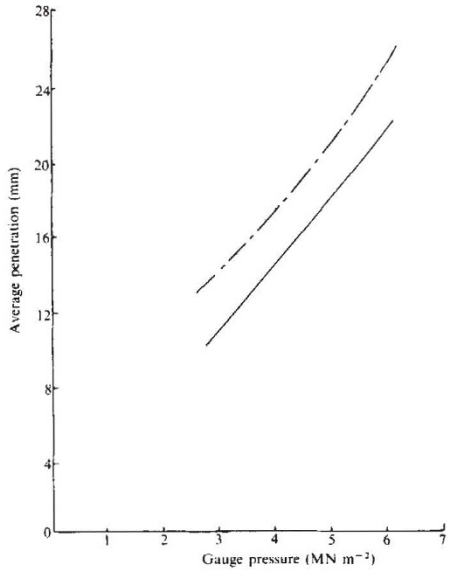

FIG. 1 Comparison between penetration of a water jet and jet containing Polyox into sandstone under varying pressure (after ref. 2). ——, Ordinary jet; — — —, with 0.1% Polyox.