Increased calcium in dystrophic muscle

SIR,—We have read with interest the article by Professor A E H Emery and Mr D Burt (9 February, p 355) on the occurrence of eosinophilic muscle fibres and increased intracellular calcium in Duchenne muscular dystrophy and in fetuses at risk for the disease and would like to comment on some of their observations and interpretations. The authors state that these features are useful for identifying dystrophic fetuses and imply that increased calcium may relate to the primary defect, but we feel that caution is needed before these conclusions can be reached.

In our experience, and as is also apparent from fig 2a in the article, the distribution of eosinophilic fibres is not random and they can occur in groups in both controls and at-risk fetuses. This is unlike Duchenne muscular dystrophy itself, where these fibres are usually scattered throughout the biopsy specimen or at the most occur in very small groups. This clustering also hampers quantification, which then entails sampling large areas but unfortunately no indication of how the quantification was carried out has been given in this article.

Preservation is also an important factor and is influenced not only by the length of time between abortion and processing of the tissue but also by the time between prostaglandin induction and abortion. Samples sent to laboratories from other centres often take several days to arrive and we have noticed a marked increase in eosinophilic fibres in such material from control and at-risk fetuses and also in some fetuses that have remained in utero for many hours after prostaglandin administration. The possibility that an increase in the number of eosinophilic fibres in fetal muscle is related to preservation and not to dystrophy must therefore be considered. This, however, does not imply that they are not an important feature of biopsies from patients with Duchenne muscular dystrophy.

With regard to the presence of calcium in eosinophilic fibres, these fibres stain heavily with most histological and histochemical stains applied to muscle and the possibility that they bind all dyes non-specifically has to be excluded before drawing firm conclusions about the presence of excess calcium. Even if this is disregarded, it must be remembered that fetal muscle is post-mortem material and an early event in cell damage and cell death in many tissues is an accumulation of calcium, irrespective of whether it is normal or diseased. Furthermore, the effect of prostaglandins may vary from fetus to fetus and cannot be ignored. The presence of increased calcium in fetal muscle is therefore not surprising and its significance in potentially dystrophic fetuses dubious.

The presence of increased calcium in biopsy specimens from Duchenne patients is also to be expected in a condition with such abundant muscle damage. Calcium accumulation in muscle occurs in a variety of abnormal situations, such as nutritional deficiencies, denervation, and ischaemia and in several neuromuscular disorders, indicating that it is a non-specific effect and secondary to any genetic defect. The light microscopical stains at best are only sensitive down to a concentration of 1-2 mmol/l and an increase in calcium of this magnitude will detect only fibres that are already well advanced towards necrosis, even if this is not morphologically detectable with the light microscope. Results with such techniques cannot therefore be used as evidence for early involvement of calcium in dystrophic muscle. We were also surprised that a 7-year-old child should appear as an early case of Duchenne muscular dystrophy as not only are clinical signs usually present before this but striking pathological changes in the muscle can be found very early in the disease before symptoms are apparent.

While increased calcium probably occurs early in damaged muscle (a view that our own data on elevated intranuclear calcium in Duchenne muscular dystrophy and carriers

support) and probably influences several cellular activities, there is at present no evidence to suggest that it precedes other abnormalities or that it occurs in the absence of other defects in Duchenne muscular dystrophy. Alterations in calcium concentrations should therefore be considered secondary to cell damage until proved otherwise. This opinion does not detract from any possible therapeutic approach to controlling calcium influx, but it emphasises that muscle reacts to severe damage like many tissues and that by detecting differences in calcium in Duchenne muscular dystrophy we are still some way from finding the primary genetic lesion. The crucial problem in Duchenne muscular dystrophy is to identify the pathological events that calcium disturbances subsequently reflect and enhance.

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* * * We sent a copy of this letter to the authors, whose reply is printed below.—ED, *BMJ*.

SIR,—In essence Dr C A Maunder-Sewry and Professor V Dubowitz suggest that our observation of an increase in the proportion of calcium-positive fibres in muscle from male fetuses at risk for Duchenne muscular dystrophy may be secondary to cell death. We would agree that this is a possibility but feel on balance that the evidence is more in favour of these findings being of relevance in the pathogenesis of this disease.

Regarding the distribution of these fibres in fetal muscle, they generally appear randomly distributed and the grouping shown in our photomicrographs was specially chosen for illustrative purposes so that comparisons between serial sections with different staining techniques would be more convincing. In each case quantification was carried out using the entire area (apart from the extreme periphery) of each transverse section and the proportion of abnormal fibres determined among 500 muscle fibres in randomly selected fields at a magnification of $\times 400$.

As stated in our paper, the method of pregnancy termination did not appear to affect the proportion of calcium-positive fibres in either control or at-risk fetal muscle. We have stored fetal muscle at room temperature and at 4°C for up to three days (the absolute maximum period between termination and our receiving a specimen) and sampling the tissue at intervals throughout this period has revealed no significant change in the proportion of calcium-positive fibres. Histochemical studies for calcium were always accompanied by studies on control sections pretreated with EDTA to remove calcium, and the fluorescence method we used is accepted as being much more sensitive and more specific than the usual histochemical methods. Of course, direct measurement of intracellular calcium would be particularly valuable in this regard and this is currently under investigation.

Finally, there are two main reasons for thinking that increased intracellular calcium is an early biochemical change and may be relevant to pathogenesis. Firstly, in at-risk fetal muscle, in which there are no morphological abnormalities or evidence of cell death (for example, necrosis and phagocytosis) as there are in muscle from affected boys, there was a good correlation between the proportion of calcium-positive fibres and other muscle fibre parameters considered abnormal in at-risk fetuses. We have so far studied 35 at-risk male fetuses and in 18 we have information

on the proportion of calcium-positive fibres. The correlations between the proportion of these fibres and mean fibre diameter was 0.43 ($p < 0.05$), the variance in fibre size was 0.44 ($p < 0.05$), and the proportion of hyaline fibres was 0.77 ($p < 0.01$). Three fetuses were considered abnormal on the basis of these parameters, and only these three and no others had a significantly increased proportion of calcium-positive fibres. Secondly, as we pointed out in our paper, many of the abnormalities in erythrocytes and lymphocytes which have recently been reported in Duchenne muscular dystrophy can be mimicked in normal cells when these are exposed to the ionophore A23187, which increases intracellular calcium.

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Improvement of anaemia in haemodialysed patients after hepatic cytolysis

SIR,—Dr P Simon and others (29 March, p 892) ascribe the improvement of anaemia in haemodialysed patients after viral or toxic hepatic cytolysis to production by the injured liver of erythropoietin. May I postulate an additional mechanism?

In chronic renal failure the erythrocyte membrane is low in cholesterol and as a consequence there is increased osmotic fragility of the erythrocyte.¹ An increase in cholesterol in the erythrocyte membrane may result in a decrease in osmotic fragility and hence prolonged survival. Such a mechanism is supported by the improvement in erythrocyte survival reported in a patient with hereditary spherocytosis who developed nephrotic syndrome.² During periods when his nephrotic syndrome was present and serum and erythrocyte membrane cholesterol levels were elevated his haemolysis decreased, while haemolysis increased when his nephrotic syndrome resolved and serum and erythrocyte membrane cholesterol levels had returned to normal. Patients with various forms of liver disease have an increased content of cholesterol in their erythrocyte membrane. The improvement of anaemia in patients on haemodialysis with hepatic cytolysis may also be due to prolonged erythrocyte survival as a consequence of increased erythrocyte membrane cholesterol.

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¹ Sasaki S. *Osaka City Med J* 1975;23:85-98.

² Takase A, Matsuda I. *Jpn J Clin Hematol* 1975;16:35-8.

Treating fungal infections

SIR,—We read with interest your leading article on treating fungus infection (8 March, p 668) and we would like to add our experience of managing fungal peritonitis in patients on chronic peritoneal dialysis.

We have encountered this complication in seven patients on continuous ambulatory peritoneal dialysis and in four on chronic intermittent peritoneal dialysis. Seven of these were *Candida* species, two *Fusarium*, one *Mucor*, and one *Trichosporon*. In seven of the