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 deficiences, 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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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

11 cases, initial Gram stain of the dialysate identified a fungus, indicating the value of this test for diagnosis. This finding is of further importance because it takes two to three days for a fungus to grow on culture. Nine of the 11 patients were treated with continuous peritoneal lavage with dialysate containing either amphotericin B (5 mg/l), or 5-fluorocytosine (50-1000 mg/l), or miconozole (20 mg/l). In seven of these nine patients this treatment proved unsuccessful and the infection was controlled only after the removal of the permanent peritoneal catheter. It is of interest that three of these patients (one with *Candida* and two with *Fusarium* peritonitis) were not given any systemic antifungal agent after the catheter had been removed. Five patients were able to continue or recommence chronic peritoneal dialysis following reimplantation of a new catheter after a period of two to eight weeks, during which time they were maintained on haemodialysis. Four patients were transferred to haemodialysis and one subsequently received a successful kidney transplant. Two patients who had bowel perforation—that is, simultaneous infection with a fungus and another enteric organism such as *Bacteroides*, *Pseudomonas*, or *Proteus*—died.

Our experience indicates that removal of the permanent peritoneal catheter and temporary discontinuation of peritoneal dialysis are very important steps in the management of fungal peritonitis. Its prognosis is favourable so long as it is not a manifestation of faecal peritonitis.

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SIR,—In your leading article "Treating fungal infections" (8 March, p 668) the comment was made that the imidazoles require further systemic evaluation and that the efficacy of miconazole given parenterally is still unclear.

With regard to the latter point, the results of series of patients treated with miconazole intravenous solution for coccidiodomycoses,[1] oesophageal candidiasis,[2] chronic mucocutaneous candidiasis,[3] and fungal meningitis[4] indicate both the efficacy and the relatively low toxicity of this agent.[5] A recent publication[6] which evaluated miconazole intravenous solution in systemic candidiasis arising in 37 patients receiving therapy for advanced malignancy suggested that eradication of the fungus is achieved at a rate comparable to that observed with amphotericin, and overall the drug was well tolerated.

Janssen Pharmaceutical Ltd has recently completed a one-year period of postmarketing surveillance designed to monitor the efficacy and safety of miconazole intravenous solution in routine use. A total of 89 case record forms have been received and the scheme is being continued. Although patients were often seriously ill with severe underlying disease, the fungus was eliminated in over half of the cases of proved systemic candidiasis, in agreement with the findings of other workers.[6] Patients whose infection did not respond were often treated late in the course of their disease, underlining the point made about problems in diagnosis, or did not show objective evidence of systemic fungal infection before treatment was begun. As with other reports on this compound, no renal or hepatic toxicity was observed and the drug was reported to be "well tolerated" in most patients.

Although we agree that intravenous miconazole is a relatively new agent, we believe that a considerable body of evidence of its efficacy has accumulated that is sufficient to recommend its use in many instances where the toxicity associated with amphotericin precludes its use. This is particularly important in the less specialised hospital unit where sophisticated renal and hepatic monitoring may be less easily achieved.

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Fasting blood glucose concentrations and treatment of maturity-onset diabetes

SIR,—Dr R B Paisey and colleagues (1 March, p 596) report close correlations between the glucose concentrations in fasting capillary blood samples of maturity-onset diabetics collected at home and seven blood glucose concentrations taken during the day. They comment that it might be reasonable to monitor diabetes control by measurement of either fasting or mid-morning blood glucose values. Because of the feasibility of treating maturity-onset diabetics with sulphonylureas aiming to lower the fasting plasma glucose to normal,[1] and the relevance of this index of control,[2] we have studied the precision of measurement of fasting blood glucose concentrations of maturity-onset diabetics at home and in general practice clinics.[3]

The mean fasting plasma glucose of all 84 maturity-onset diabetics from three general practices was 8·1 mmol/l, and the day-to-day repeatability of home samples (either with stored Reflotest strips in dessicant bottles or blood samples in vacuum collector bottles) was similar to that of venous samples measured in the clinic (±1SD, 0·8 mmol/l (14·4 mg/100 ml)).[3] This small variability suggested that it would be feasible to aim to lower the fasting blood glucose to under 6 mmol/l (108 mg/100 ml). This was attempted in 71 of the patients studied over six months, and whereas initially 29 (41%) patients had a fasting plasma glucose of under 6 mmol/l, by increasing the chlorpropamide dose and by dietary advice the fasting plasma glucose was reduced to under 6 mmol/l in 54 (76%) patients.[4] No episodes of hypoglycaemia were induced by increasing the sulphonylurea dose (and in the six-month follow-up only two patients had hypoglycaemic episodes—both induced by losing over 8 kg weight, because of strict dieting and disseminated cancer respectively). Forty-one of the 54 patients whose fasting plasma glucose fell below 6 mmol/l maintained this degree of control, as determined by repeat fasting blood glucose concentrations in the general practice clinic three and six months later. There are now nine general practices in Oxford monitoring the diabetes of their maturity-onset patients by the means of the two criteria of fasting blood glucose concentration (measured with Reflotest on a Reflomat meter) and body weight.

Although Dr Paisey and his colleagues reported that the haemoglobin A_1 concentrations in their patients were normal if the fasting blood glucose was <7 mmol/l (126 mg/100 ml), we found raised concentrations in many maturity-onset diabetic patients who achieved a fasting blood glucose of under 6 mmol/l (mean haemoglobin A_1 concentrations 8·9%, normal range 6-8%, Bio-Rad columns). This would be apposite in relation to these patients still having a raised fasting blood glucose (mean 5·7 mmol/l (103 mg/100 ml), normal range 3·5-5 mmol/l (63-90 mg/100 ml)), as well as sulphonylurea-treated maturity-onset diabetic patients still having raised postprandial glucose concentrations even with entirely normal fasting blood glucose concentrations.[1]

Nevertheless, the simplicity of assessing control of maturity-onset diabetic patients by means of the easily understood criterion of the fasting blood glucose concentration provides, together with the body weight, an effective and acceptable mode of monitoring therapy.

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Lithium and the kidney

SIR,—Recent information prompts us to add a postscript to our paper "Renal function after long-term treatment with lithium" (2 June 1979, p 1457) and to emphasise again the importance of keeping plasma lithium concentrations as low as possible.

Possible long-term kidney damage following lithium treatment is invariably discussed in relation to polyuria, considered by some to be inevitable in many lithium patients. Widespread changes in renal function are not seen in our population of 120 patients, who have received lithium for up to 12 years.[1 2] The incidence of so-called polyuria (<3½ litres/day) was 5% (six patients); in some centres the incidence is up to 40%. Many patients with renal histopathological changes[2] had suffered previous episodes of acute lithium toxicity, which is virtually unknown in our patients.

There is no strong evidence confirming that polyuria and renal histopathology are related by common mechanisms. Polyuria may be due to many factors. Early fears of toxic effects of lithium led physicians to insist on increased fluid intake during lithium therapy. The habit so formed, or lack of counter-instruction, would result in resetting of thirst mechanisms[3] and to permanent overhydration. Similarly the taste of lithium, secreted in saliva, might be a stimulus to drink in order to remove the taste. The histological changes reported during long-term lithium therapy might be due to acute nephrotoxicity during discrete episodes of lithium intoxication. Alternatively, histological change might result from long-term polypharmacy, common in patients with chronic psychiatric conditions.

We have stressed[1] that the lithium concentrations used in many biochemical studies in vitro were very high (compared with a normal plasma lithium concentration of 1 mmol/l (0·7 mg/100 ml)). However, our Scandinavian colleagues have shown that peak urinary lithium concentrations exceeding 65 mmol/l (45 mg/100 ml) occur in their patients during