

clinicians have in deciding a cut-off level for management Ales and Santini need to ponder the principal finding of Farmer et al's study,³ which was that maternal glucose tolerance is a continuum relating to morbid risk to the fetus.

Another approach to evaluation of the risks of gestational glucose intolerance and hence to the justification for screening—namely glucose tolerance assessment after unexplained stillbirth—is also ignored. This may be justifiable on the grounds that grief induces sufficient lasting stress to the extent that around two months after the event the intravenous glucose tolerance test is unreliable. However, Ales and Santini may have been unaware of the data which show that about one-fifth of women have abnormal intravenous glucose tolerance 6–8 weeks after unexplained stillbirth.⁴ A further omission was their failure to comment on the non-reproducibility of the oral glucose tolerance test in pregnancy.^{5,6}

Their conclusion that data to support universal screening are limited is correct, if circumspect; but we do not agree that "a more restrained approach than universal screening may be appropriate". Metabolic error associated with neonatal hazard is asymptomatic, so case selection is not an option. Diabetogenic fetopathy must be the result of failure to metabolise mixed food properly. Postprandial blood sugar measurement is an option and is direct, simple, and inexpensive, although the meal should be standardised.⁷ At a time when studies are beginning to show that blood sugar concentrations alter a standardised meal in pregnancy relate not only to birthweight but also to perinatal morbidity,⁸ it would be a pity indeed if Ales and Santini were to weaken the argument of clinicians seeking to provide screening. For the mother, Ales and Santini do concede that gestational glucose intolerance identifies women at risk of subsequent diabetes mellitus and note that on-going dietary treatment after the index pregnancy later led to a much lower than expected incidence of overt diabetes mellitus.⁹

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SIR,—Dr Ales and Dr Santini conclude that there is no evidence for the need for universal screening for gestational diabetes, after studying the maternal and fetal risks from gestational diabetes and the possible benefits of treatment. They briefly mention a study of Pima Indians.¹ This investigation showed that offspring of gestational diabetic mothers have increased risk for obesity and glucose intolerance in later life.

It cannot be proven whether this effect is related to the intrauterine diabetic milieu or to a genetic predisposition. However, in rats in which we induced "gestational diabetes", we found that transmission of diabetes is not genetic but due to the diabetic intrauterine milieu. Diabetes was transmitted only through the female line and not through the paternal line.² Moreover the intrauterine diabetic milieu induces a defect not only at the level of the insulin-producing β cells but also at the level of insulin receptor function, resulting in insulin resistance.³

The offspring of diabetic male mice are at greater risk of becoming diabetic.⁴ These data do not contradict the results in Pima Indians or our data in rats. Indeed, in our studies, we are dealing with gestational diabetes. Immunological changes, as shown by eosinophilic infiltration in the fetal pancreatic islets, are only present in type I diabetes and not in infants born to gestational diabetic mothers.⁵ It is possible that these immunological changes are protective in the development of diabetes in later life.

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PROTEASE ANTIGENS FROM HOUSE DUST MITE

SIR,—A cDNA clone coding for one of the major antigens from the house dust mite *Dermatophagoides pteronyssinus*, originally called *Der p I* but now referred to as *Der p III*, has been isolated and sequenced.¹ Search of protein sequence data banks showed that this clone coded for a putative protease belonging to the cysteine family and that *Der p III* had extensive homology with the mammalian enzymes cathepsin B and H, and the plant enzymes papain and actinidin. To confirm these observations, we purified *Der p III* from spent growth medium obtained after culturing *D. pteronyssinus* by a combination of physicochemical procedures.² Its proteolytic activity was measured by release of soluble peptides from the insoluble general protease substrate "Azocoll".³ These experiments showed that *Der p III* was active and that prior activation with a reducing reagent was required for expression (table 1). On the basis of these findings, protease studies were done on extracts of house dust collected from the homes of mite asthmatic volunteers. Cysteine protease activity correlated with levels of *Der p III* measured by radioimmunoassay (table 1).

During these studies it became apparent that proteases distinct from the cysteine dependent *Der p III* were also present, both in extracts of spent growth medium⁴ and in house dust. We are characterising the proteases in spent growth medium and have shown that proteins binding to a soybean trypsin inhibitor affinity-chromatography matrix were allergenic by direct radioallergosorbent assay. A mite protein was isolated from spent growth medium on ion-exchange chromatography and high-performance liquid chromatography, and its N-terminal sequenced. The sequence demonstrated homology with that for an allergen isolated from *D. farinace* (table 1), designated *Der f III*.⁵ However, sequence analysis of *Der p III* and *Der f III* with the protein identification resource of the National Biomedical Research Foundation showed that both proteins had significant N-terminal

TABLE 1—CYSTEINE DEPENDENT AND INDEPENDENT PROTEASE ACTIVITY ASSOCIATED WITH PURIFIED *Der p III* AND HOUSE DUST SAMPLES

	Protease activity associated with:		Pearson's correlation between <i>Der p III</i> concentration and protease activity in dust (n = 126)
	Purified <i>Der p III</i> (units/mg protein)	House dust (mean units/g dust extracted, [SE] n = 126)	
Cysteine			
Non-activated	98	146 (13)	r = 0.44, p < 0.001
Activated	1312	184 (14)*	r = 0.52, p < 0.001

*Represents both activated and non-activated protease in dust samples.

1 unit = optical density at 520 nm $\times$ 1000 per min per mg of purified *Der p III* or g house dust extracted.

TABLE II—HOMOLOGY BETWEEN *Der f* III, *Der p* III, AND SERINE PROTEASES CHYMOTRYPSIN AND TRYPSIN

	Sequence (1 on left, 18 on right)																		Homology (%)
<i>Der f</i> III ¹	Ile	Val	Gly	Gly	Val	Lys	Ala	Leu	Ala	Gly	Asp	-X-	Pro	Tyr	Glu	Ile	Ser	Leu	..
<i>Der p</i> III	Ile	Val	Gly	(Ser)	Glu	Lys	Ala	Leu	Ala	Gly	Glu	-X-	Pro	Tyr	Gln	Ile	Ser		71
Chymotrypsin (crayfish) ²	Ile	Val	Gly	Gly	Thr	Asn	Ala	Pro	Arg	Gly	Lys	Tyr	Pro	Tyr	Gln	Val	Ser	Leu	50
Trypsin (hornet) ³	Ile	Val	Gly	Gly	Thr	Asp	Ala	Val	Leu	Gly	Glu	Phe	Pro	Tyr	Gln	Leu	Ser	Phe	45

Homologous residues are boxed. X = residue could not be ascertained; residue in parenthesis indicates probable identity. Both *Der p* III and *Der f* III sequences had homology with vertebrate serine proteases of about 40%. Homology expressed as percentage of known *Der f* III sequence for corresponding residues (1–18 for chymotrypsin and trypsin, and 1–17 for *Der p* III).

homology with vertebrate and invertebrate serine proteases such as trypsin and chymotrypsin (table II).

These data indicate that mite extracts contain proteolytically active antigens belonging to the serine and cysteine protease families and, given the observations about the sensitisation of individuals to both types of enzymes,^{8,9} our findings suggest that proteolytic activity per se is an important feature contributing to mite allergic disease in susceptible individuals. In addition, antigens associated with the faecal particle other than the group I and III antigens may well be enzymes involved in digestion.

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adult females. Twenty-four patients were shown once how to measure out this length of ointment and then asked to squeeze out this amount on their index finger from a 100 g tube of betamethasone valerate 0.025% ointment. The expressed ointment weighed, for males, 0.49 g (0.09) and, for females, 0.43 g (0.09). In adults one fingertip unit (2.5 cm [0.22], n = 24, about one inch) of ointment from a standard 5 mm nozzle therefore represents approximately 0.5 g (males) or 0.4 g (females). Roughly, therefore, for a 5 mm diameter tube nozzle, 2 or 2.5 fingertip units represents 1 g.

The use of a fingertip unit does have some inaccuracies and variability, but has the advantage of being understandable and meaningful to patients and doctors. This simple method has potential in ensuring that patients do not use excessive amounts of topical steroids, and should also help clinicians prescribe appropriate amounts of topical drugs.

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LACTATIONAL HYPOMAGNESAEMIA

SIR,—Magnesium deficiency during lactation is well known in cows¹ but only one human case has been reported. We report another case.

A 24-year-old woman was admitted to the cardiac intensive care unit of the Government Medical College, Nagpur, because of rapidly progressive weakness of all her limbs for the past 4 h. The patient had had a normal full-term hospital delivery 15 days before admission and had undergone tubectomy 5 days before admission. On the day of admission the weakness started initially on the left side and then involved both the right arm and leg within 30 min. There was no history of sensory disorders, cranial nerve involvement, fever, loose motions, vomiting, increased urine output, diabetes mellitus, or relevant drug use. She had three children, aged 5 years, 3 years, and 20 days old, and had breast fed them all. She had no similar complaints during previous lactations. Pulse was 62/min and irregular. Other vital signs and breasts were normal. She was conscious and anxious. Muscle bulk was normal; there was claspknife spasticity in all four limbs, deep tendon reflexes were exaggerated, plantar reflexes were indeterminate, and abdominal reflexes were absent. Electrocardiogram revealed evidence of hypokalaemia and multiple ventricular premature beats. Haematological examination was normal. Serum magnesium was 0.5 mmol/l (normal 0.8–1.7) and milk magnesium was 4.9 mmol/l (normal 1–6). Urinary magnesium was 0.8 mmol/l (normal 45–123). Serum calcium was 2.8 mmol/l and phosphate 1.1 mmol/l. Serum parathormone was normal. Urinary potassium was 36 mmol/l.

The patient was treated with an intravenous magnesium 2 g bolus and was then put on magnesium sulphate, 2 g orally once a day. She also received intravenous lignocaine and 'Isolyte E'. After magnesium and potassium replacement the ventricular premature beats disappeared; power improved to grade 5 (Medical Research Council scale); and deep tendon reflexes returned to normal. Serum magnesium increased after therapy to normal (1.1 mmol/l). She continues to breastfeed the child.

"FINGERTIP UNIT" IN DERMATOLOGY

SIR,—Prescribing in dermatology is inaccurate in that usually neither the patient nor the doctor knows how much cream or ointment should be applied. One manufacturer suggests that 3 cm of ointment squeezed from a tube with a 5 mm diameter nozzle will be about 1 g. Unfortunately the concept of 3 cm is confusing to some patients and our measurements show that 3 cm of ointment from a 5 mm nozzle weighs about 0.6 g. A new concept of a "fingertip unit" is understandable by patients and relevant since patients usually squeeze ointment from a tube held in one hand onto the fingertip of the opposite hand, usually onto the index finger.

The average length of the distal phalanx of the index finger, taken from the distal crease to the end of the finger on the palmar aspect is 2.5 cm (SD 0.11, n = 12) for adult males and 2.3 cm (0.10, n = 12) for

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