no known unique quality. Historically a major commitment to the cotton industry has been a feature of the high prevalence area, yet other towns also associated with the cotton industry—for example, Rochdale—have low prevalences.

We are most grateful to the radiologists, radiographers, and x-ray records staff in the towns surveyed. Their kindness and help made the survey possible. The study was funded by a grant from the Department of Health and Social Security.

SHORT REPORTS

Prenatal chromosomal analysis of fetal blood obtained at fetoscopy

Cultivation of amniotic fluid cells for prenatal diagnosis may take several weeks. But the fetal karyotype in a mother who presents in an advanced stage of pregnancy may need to be determined within a few days. We describe here a method of doing a fetal chromosomal analysis within 72 hours. Fetoscopy samples of mixtures of fetal blood and amniotic fluid^{1 2} are cultured by a modified technique of micro-culturing whole blood. We also report our first clinical application of the method.

Patients, methods, and results

The fetuses of 14 women in the 16th to 23rd week of gestation were studied. Thirteen of the women had been admitted for therapeutic abortion. In one of them fetal trisomy 18 had been diagnosed by amniocentesis. The remaining woman was a case for diagnosis. She had had a child with Down's syndrome. In the 22nd week of her gestation it became obvious that a second attempt to culture amniotic fluid cells had failed. Since she refused to accept the risk of having another affected child she agreed to prenatal diagnosis by analysis of fetal blood obtained at fetoscopy.

The site of the placenta and the position of the fetus were determined by ultrasound immediately before fetoscopy. Under direct visual control (Dyonics Needlescope) a placental vessel was punctured without any attempt at cannulation. A sample (2 ml) of blood mixed with amniotic fluid was aspirated and immediately transferred to a glass tube containing 3.5 IU heparin. After centrifugation at 125 g for 10 minutes the supernatant was discarded and the cell button was deposited in a 6-ml Falcon plastic tube containing 1 ml McCoy's 5a medium (Flow Laboratories) with 20% fetal bovine serum and 0.026 ml phytohaemagglutinin supplemented with streptomycin and benzyl penicillin. After incubation for 65–70 hours at 37°C cell growth was stopped by adding colchicine in a final concentration of 0.125 µg/ml. After hypotonic treatment with potassium chloride 0.075 mmol/l for 10 minutes at room temperature the cells were fixed in three parts methanol and one part acetic acid. Slides were prepared with the standard air-drying technique. The chromosomes were stained with the trypsin-Giemsa banding technique according to Seabright³ as slightly modified by Özkinay and Mitelman.⁴

Analysable metaphase plates were obtained in 12 out of the 14 cases (table). The fetus of the woman who had borne a child with Down's syndrome had a normal karyotype (46,XY) and at birth was normal, as predicted. The diagnosis in the case of fetal trisomy 18, established by

Chromosomal analysis in fetoscopy samples*

Fetoscopy case No	Gestational age (weeks)	Amount of blood (µl)	No of analysable metaphases	Karyotype
1	18	130	2	46,XY
2	16	70	3	46,XY
3	19	<10	0	
4	19	400	58	46,XX
5	22	280	>100	46,XX
6	23	90	73	46,XX
7	19	20	1	47,XY,+18
8	19	<10	0	
9	17	30	7	46,XY
10	18	40	4	46,XY
11	22	50	>100	46,XX
12	18	280	>100	46,XX
13	22	130	24	46,XX
14†	22	520	>100	46,XY

*Mixtures (2 ml) of fetal blood and amniotic fluid. The percentage of fetal red blood cells (Kleihauer technique) was 80–90% in samples in cases 1 and 4; the remaining samples contained >95% fetal red blood cells.

†Diagnostic fetoscopy in a woman who had borne a child with Down's syndrome.

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amniocentesis, was verified in the fetoscopy sample. Examination of the fetuses after the abortion showed that sex prediction was correct in all of them.

Comment

The essential step in the procedure seems to be centrifuging the mixed sample before culture. Our first attempts to culture the cells without previous centrifugation usually failed. Pure fetal blood uncontaminated with amniotic fluid⁵ would certainly be more suitable for culture than a mixed sample. But sampling pure fetal blood at fetoscopy requires cannulation of the placental vessel with a risk of penetrating the chorionic plate and consequent admixture of maternal blood in the sample. The procedure proved diagnostically reliable in the clinical case. Within three days we could tell the woman that the results of our chromosomal studies were normal. This was later confirmed by the birth of a normal infant.

Although amniocentesis remains the method of choice for prenatal diagnosis of chromosomal abnormalities the procedure described may prove a useful alternative when it is impracticable to wait about three weeks for the results of amniotic fluid cell culture.

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"Catabolic" loss of body protein after human liver transplantation

Little is known about the metabolic response to orthotopic liver transplantation. The trauma and length of the operation would be expected to evoke a major "catabolic" response in normal patients. Furthermore, the routine postoperative high doses of prednisolone to prevent graft rejection would be expected to increase catabolism further. On the other hand, such a response, with increased urea nitrogen excretion in the urine, would be suppressed if the transplanted liver failed to function normally since protein is oxidised and urea synthesised by the liver. Moreover, patients depleted of protein,

as many transplant recipients are, do not show the usual catabolic response to trauma.¹ Most patients in the present study had not been given dietary protein for the first 10 days after surgery, since the postoperative regimen until recently comprised intravenous dextrose and electrolyte infusions alone. Thus in the patients with normal renal function the rate of loss of urea nitrogen in urine could be used to calculate the rate of protein catabolism. In such circumstances the rate of urea excretion may also be used as a measure of liver function.²

Patients, methods, and results

A group of 42 patients who survived a minimum of 10 days after orthotopic liver transplantation were studied. The pretransplant diagnosis was end-stage cirrhosis in 18 (cryptogenic 6, alcoholic 3, biliary 9), hepatocellular carcinoma in 12 (with cirrhosis in 3), cholangiocarcinoma in 5, and chronic active hepatitis in 3. Postoperative management included intravenous fluid and electrolyte replacement and immunosuppression with hydrocortisone 500 mg daily for the first three days followed by prednisolone 200 mg daily, the dose then being gradually reduced. For the first three days cyclophosphamide 100 mg/day was also given, thereafter being replaced by azathioprine 150 mg/day. Urea and creatinine content in daily urine collections were measured for 10 days by routine (Technicon) autoanalysis. Creatinine clearance was normal in all but five patients, in whom it was reduced to below 50 ml/h. The rate of protein oxidative loss, or catabolism, was calculated from the formula:

$$\text{catabolic rate (g body protein/day)} = 6.25 \left(\frac{28 \times \text{UU}}{1000} + 2.0 + 1.5 \right)$$

where UU=rate of urine urea excretion in mmol/24h, 28=molecular weight of nitrogen contained within urea, 2.0=standard allowance for non-urea nitrogen excretion in urine, and 1.5=standard allowance for loss of nitrogen in skin and faeces.³

Finally, protein requirement was estimated from the relationship:

$$\text{protein requirement (g/day)} = \frac{\text{catabolic rate}}{\text{efficiency of utilisation of dietary protein}}$$

The mean loss of nitrogen in the form of urinary urea was high, rising to a maximum of 12.5 g/day on the seventh postoperative day (figure). The variation was considerable, with some patients excreting almost maximal quantities of 35 g N/day, representing the catabolism of 219 g protein/day. On the other hand, the excretion rates were low in five patients with renal impairment.

Mean (±SD) daily rate of urinary urea excretion in 42 patients and resultant catabolic rate of protein (g/day).

Comment

Recognition that protein catabolism can be severe is important since protein depletion is known to be associated with reduced host defence and increased mortality.⁴ The combined effect of further depletion (due to catabolism) and the postoperative immunosuppressive treatment must contribute to the high incidence of fatal septicæmias in these patients. Loss of body protein may be reduced by giving intravenous amino-acid solutions. The efficiency of utilisation of protein, however, is low under such circumstances—usually under 50%—and, therefore, the calculated requirement would be high (a mean of about 200 g/day for this group of patients). Alternatively, the requirement may be reduced if catabolism is suppressed. Catabolism

can be reduced by infusing concentrated glucose solutions (for example, glucose 600–700 g/day) together with enough insulin to maintain normoglycaemia.⁵

The high protein oxidation and urea excretion rates indicate that liver function in our patients was generally very satisfactory, and therefore they should be able to tolerate normal or even high protein intakes.

We thank the members of the King's College Hospital Liver Unit transplant rota who supervised the metabolic care of these patients, and the sisters and nursing staff of the intensive care unit, New Addenbrooke's Hospital, Cambridge, who completed the urinary collections.

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ONE HUNDRED YEARS AGO Dr William G Porter has presented a case (*Transactions of the College of Physicians of Philadelphia*, 1879) which has a medico-legal interest in its relation to the question of decomposition as a means of determining the time of the death in any given case. The patient was an Englishman, fifty-eight years old, of stout build, of temperate habits, though a large eater; he was a healthy man, so far as was known, until his last illness attacked him. Dr Porter was called at 5 AM, October 4th, to prescribe for the relief of excruciating epigastric pain which had supervened after several days of nausea, with occasional vomiting. Morphine, given hypodermically, controlled the pain; and other measures were adopted to palliate the attack and to prevent its recurrence. Moderate fever developed, and in the course of the day several large soft bilious stools were passed. At midnight, there was more pain, still referred to the epigastrium; the skin was warm, but the patient was pulseless at the wrist; full collapse developed presently, and death ensued at about 1 AM, October 5th. An undertaker was immediately summoned, and the body was placed in ice within two hours after death. At eight o'clock in the morning, the body had swollen "enormously"; at nine, the corpse, by its distension, had lifted the lid of the ice-box several inches, carrying with it a layer of ice that covered it. The whole surface of the body was jaundiced. The features were distended beyond recognition, and the face was smeared with clotted blood which had been forced from the bloated mouth and nostrils. When at this time (eight hours after death) the usual *post mortem* incision was made in the median line of the body, air without appreciable odour escaped from the cellular tissue; the tissues receded from the knife as if they had been stretched to the utmost. A large amount of air, also without odour, escaped from the peritoneal cavity, although there was no peritonitis. The liver, kidneys, and spleen were emphysematous and buoyant. The heart was empty, and its tissue was friable. There was no odour of putrefaction. (*British Medical Journal*, 1880.)

Correction

Impaired hearing in the elderly

In this article by Mr G W Crammond and Dr Patricia Gabb (1 March, p 612) the degree ChB was wrongly attributed to Mr Crammond.