We recently encountered an example of this condition in a 50 year old Caucasian man who presented, via casualty, to the local infectious diseases unit. He gave a five day history of fever followed by non-pruritic jaundice, malaise, and both intermittent chest and abdominal pain. He was also anorectic, and had lost one stone in weight. Hepatitis was diagnosed. Despite initial treatment at home, with a combination of amoxycillin and clavulanic acid, from the third day of his illness, he had continued to deteriorate and at the time of presentation was drowsy with an expressive dysphasia.

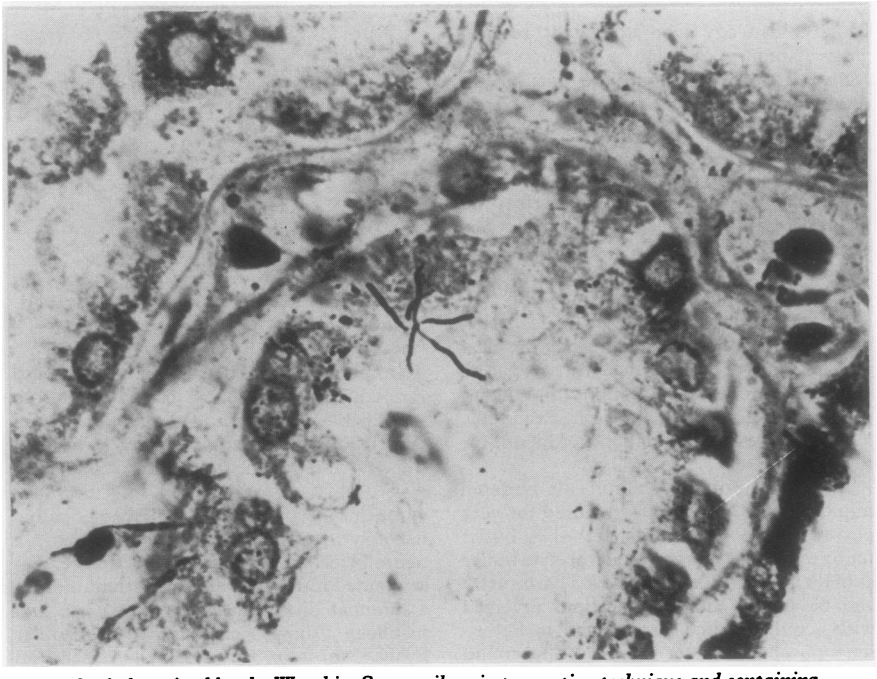
His blood count showed a haemoglobin concentration of 7·7 g/dl, a white cell count of $6{\cdot}5 \times 10^9$/l, and a platelet count of 20×10^9/l. The blood film contained nucleated red cells and microspherocytes. The working diagnosis at this time was of leptospirosis: according to the patient's relatives, he had been working in a drain a week before the onset of symptoms. Other investigations showed increased creatinine and urea concentrations with an abnormal coagulation screen, suggesting disseminated intravascular coagulation (prothrombin time 16·5 seconds; normal range 10–13 seconds: kaolin-cephalin clotting time 50 seconds; normal range 27–37 seconds: thrombin time 12 seconds; control 14 seconds, normal range control $\pm 2{\cdot}5$: fibrinogen concentration 1·8 g/dl; normal range 1·6–3·9: and D-dimers of 8 μg/ml; normal range $< 0{\cdot}25$ μg/ml), although the possibility of TTP was considered in view of the persisting neurological signs. The bilirubin was raised at 101 mmol/l and liver enzymes were raised. Blood cultures and hepatitis serology were negative, in particular the leptospira serology was reported as being negative at a titre of less than 10. A chest *x*-ray picture and electrocardiogram were normal, but an electroencephalogram did show a diffuse abnormality affecting the cerebral hemispheres.

Treatment was in the form of vitamin K, platelet concentrate, intravenous fluids and blood, fresh frozen plasma, and antibiotics (benzyl penicillin 4 MU every six hours, and gentamicin 120 mg every eight hours). The patient continued to deteriorate over the next 48 hours, despite the addition of high dose prednisolone for possible TTP. He was therefore transferred for plasma exchange at another hospital. At this time his coagulation profile was becoming more normal (prothrombin time 18 seconds, kaolin-cephalin clotting time 37 seconds, fibrinogen concentration 2·9 g/l, and D-dimers of 1 μg/ml), but there was evidence of a microangiopathic haemolytic anaemia with gross red cell fragmentation. The haemoglobin concentration was 10·3 g/dl and the platelet count 30×10^9/l. This combination of features was now suggestive of a diagnosis of TTP. A computed tomogram showed no intracerebral pathology.

Despite a two litre plasma exchange[4] along with continued antibiotics, steroids, and the addition of high dose intravenous immunoglobulin there was continued deterioration and he died 48 hours after admission: a second serum sample had not been sent for leptospira serology.

The important findings at necropsy were prominent petechial haemorrhages present over the whole skin surface and on the endocardial surface of the heart. Multiple renal infarcts were present and there was severe gastrointestinal bleeding. Histological examination showed leptospira in the liver, heart, and kidney (figure) when tissue sections were examined using the Warthin-Starry silver impregnation technique. Although no organisms were identified in the brain, as this technique also heavily stained nerve cells and their processes, a mild leptomeningitis was identified on routinely stained sections. Granular hyaline thrombi were present in the small vessels of the brain, heart, lungs and kidneys. Electron microscopical examination confirmed the platelet composition of these thrombi, while a Martius Scarlet Blue stain showed only minimal amounts of fibrin.

These findings were thought to be indicative of thrombotic thrombocytopenic purpura complicating leptospirosis. The association, which has not been described before, may explain the poor response to treatment. The precipitant for this condition is probably the extensive vessel wall damage caused by leptospirosis,[5] and it therefore seems likely that TTP is a more common complication of leptospirosis than previously supposed. Coagulation disorders are well recognised in leptospirosis, but have until now been described as disseminated intravascular coagulation, rather than TTP.



A renal tubule stained by the Warthin-Starry silver impregnation technique and containing several spirochaetes.

RW LAING
Department of Neuropathology, Royal Hallamshire Hospital, Glossop Road, Sheffield, S10 2JF

C TEH
Department of Medicine and Infectious Diseases, Lodge Moor Hospital, Sheffield S10 4LH

CH TOH
Department of Haematology, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF

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Implications of delay in culturing for Campylobacter

This district has two general hospitals separated by 30 miles of winding country roads. Most of the microbiology is done at one, and the other has a small laboratory performing a limited range of tests.

We compared the effectiveness of culturing faeces for *Campylobacter* species at the small laboratory or after transport to the larger one. The logistics of specimen transport are such that most specimens are not received until mid-afternoon, too late for transport to the large laboratory. During the study period specimens were processed in the small laboratory, refrigerated overnight (at 4°C), then transported to the large laboratory at ambient temperature. In both laboratories specimens were plated directly on to selective medium (blood agar base (Oxoid) with 10% lysed blood, colistin (8 mg/l), vancomycin (3 mg/l), trimethoprim (5 mg/l)) and incubated

Culture for Campylobacter

Numbers of specimens processed at:	*Small laboratory*		
Large laboratory	*Positive*	*Negative*	*Total*
Positive	93	6	99
Negative	41	3147	3188
Total	134	3153	3287

$\chi^2 = 2084{\cdot}26$ (with Yates' correction).
$p \leqslant 0{\cdot}001$.

at 42°C in a microaerophilic atmosphere. Plates were read at 24 and 48 hours, and positive results were reported on the basis of colonial appearance and microscopic morphology. Both laboratories participate in the UK NEQAS scheme for faecal specimens.

The study period comprised the whole of 1988 and 1989, and 3287 specimens were processed in parallel. The results are shown in the table. The isolation rate of *Campylobacter* was significantly better ($p \leqslant 0.001$) from specimens processed promptly.

There are several variables here, but our culture method works satisfactorily for fresh specimens in both laboratories, and the most obvious reason for the difference is the delay in culturing specimens at the large laboratory.

Storage and transport of *Campylobacter jejuni* has been studied by Monfort *et al*,[1] who found that the organism survives in animal faeces for at least three hours at 4°C but for less than two hours at 25°C; holding atmosphere had no effect on isolation rates. Our experience is that *Campylobacter* sp survive for over a week at 4°C in Stuart's transport medium. The organism may be killed by toxic metabolites, and transport at ambient temperature may be the real problem.

Thus centralisation would result in a significant false negative rate, whereas culture at the small laboratory would yield more positive results a day earlier. Other alternatives could include using transport medium (which would entail handling the specimen almost as much as direct culture) or enrichment (which would add another day to the delay already experienced); both would add to the cost.

Our observations also have implications for provision of services or storage of specimens at weekends and Bank holidays.

CJ BUTT
Yeovil District Hospital, Yeovil, Somerset

MCJ WALE
Department of Microbiology, City Hospital, Hucknall Road, Nottingham NG5 1PB

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Flow cytometric DNA in ectopic pregnancy

The estimated incidence of ectopic pregnancy varies from 1 in 84 to 1 in 230 intrauterine pregnancies.[1] There are several maternal factors associated with ectopic implantation, such as anatomical changes in the tube involved. These factors are probably more important than the less well documented factors inherent in the embryo. According to various reports, the range of abnormal embryogenesis associated with ectopic pregnancy varies from 0% to 63·6%.[2] It is difficult to ascertain from the information available whether this abnormal embryogenesis is due to increased incidence of chromosomal aberrations. The actual incidence of chromosomal anomalies in ectopic pregnancy remains largely obscure as specimens are rarely available for karyotype analysis, or karyotype analysis cannot be carried out for technical reasons.

Flow cytometric DNA analysis is rapid and relatively simple but can only show relatively gross DNA dearrangements in cell suspensions prepared from paraffin wax embedded solid tissues. The DNA contents of individual chromosomes ranges from 4·3% (chromosome No 1) to 0·8% (chromosome No 21) when expressed as a percentage of total autosomal DNA.[3] Thus in ideal conditions with fresh tissues a DNA difference of at least 8–10% would be required to detect two mixed cell populations by DNA flow cytometry.[4] This means that DNA flow cytometry would only show DNA aneuploidy if a substantial amount of the analysed cells lose or acquire several chromosomes.

We performed flow cytometric DNA analysis of 42 randomly selected cases of conceptual mass removed from the fallopian tubes. The histological material consisted of varying amounts of trophoblastic tissue with or without gestational sac and embryo, and of maternal blood. Single cell suspensions for flow cytometric analysis were prepared as described by Schutte *et al*[5] with a few modifications. Quantitative DNA analysis was performed using an EPICS-C (Coulter Electronics, Hialeah, Florida, USA) flow cytometer. For each DNA histogram up to 10 000 nuclei were scanned. The mean coefficient of variation (CV) of 8·2% was obtained (range 4·6–12·0). The DNA index (DI) for each case was calculated. The peak having the lowest channel number was considered to represent diploid cells and to have a DI of 1·0. DNA hypoploid populations might not have been identified due to the definition of the G_0G_1 peak used and due to the use of dewaxed samples characterised by excessive cell debris. The DNA Index (DI) of a given aneuploid peak was calculated by dividing its channel number by that of the diploid cells in the same sample. Tetraploidy was considered when the G2/M peak consisted of more than 15% of the total cell count.

Tubal implantation was associated with aneuploidy in 14 (33%) cases. The DNA indices of the aneuploid peaks ranged from 1·14 to 2·17. Eight (19%) cases showed karyotype changes in the near diploid region. Six cases were tetraploid. Triploid cases (DI 1·4–1·6) were not recorded.

The results of this study support earlier reported overall incidences of cytogenetic anomalies in ectopic pregnancy.[6,7] It is quite possible that chromosomal aberrations contribute significantly to the incidence of ectopic gestation, especially when the tubes seem to be grossly normal or ectopic pregnancy is recurrent. The prevalence of DNA tetraploidy was high. No triploidy—that is, DNA aneuploidy associated with partial mole morphology—was detected.

Flow cytometric DNA analysis can be of positive use in the evaluation of ectopic pregnancies. The advantage of flow cytometric analysis over conventional cytogenetics is the simplicity of the method. At the moment DNA flow cytometry is not as sensitive as cytogenetic techniques for detecting small chromosomal changes. Nevertheless, it can provide interesting but approximate information on the DNA content of the cells under study and thus on the role of karyotype abnormalities in ectopic pregnancy.

R AINE
R KARIKOSKI-LEO
Department of Pathology,
P K HEINONEN
Department of Obstetrics and Gynecology, University Central Hospital of Tampere, Finland

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MATTERS ARISING

Monocyte esterase deficiency: familial or environmental?

A recent report by Markey *et al* presents some intriguing results concerning monocyte esterase deficiency and malignant neoplasia.[1] They found a significant deficiency in 0·8% of blood donors, in 3·9% of patients with malignancies, and in 1·7% of patients with non-malignant diseases. Their method for measuring monocyte esterase deficiency was the Technicon Hemalog D automated white cell differential counter. This system uses the presence of esterase in monocytes as a means of distinguishing monocytes from neutrophils. The Hemalog D has been in use world wide since 1974, but has mostly now been phased out by the more recent Technicon H-6000 and H-1 systems. These current systems do not use esterase staining, and thus make the study performed by Markey *et al* difficult to repeat. Two other semiautomated methods for the measurement of monocyte esterase offer possible substitutes. Markovic *et al* report an image processing system which measures the esterase of blood cell smear preparations.[2] My laboratory recently reported an adaptation of the Hemalog D chemistry for use on the current Technicon H-1 system.[3] Our method, like that in use with the Hemalog D, stains cells in suspension using a whole blood sample.

The observations by Markey *et al*, while intriguing, do require further experimentation to test their hypothesis that "severe diminution in monocyte esterase activity, which occurs in an inheritable form and is relatively common, may at least in a minor