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 ($p \leq 0.001$) 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.

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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 rearrangements 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 G₀G₁ 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.

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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

way, predispose to certain malignant neoplasias." Although these authors attempt to exclude external causes for suppression of monocyte activity by comparing patients with malignancy with other patients, this may not be sufficient as a control. Monocyte esterase is easily inhibited by several pharmacological and environmental factors. Organophosphates, used as a component in insecticides, are potent inhibitors of monocyte esterase.⁴ Monocyte esterase has been shown to be a useful measure of occupational exposure to organic compounds in plastics manufacturing.⁵ The excess in monocyte esterase deficiency in patients with cancer observed by Markey *et al* could be due to differences in drug exposure compared with patients who do not have cancer. They were able to report familial studies in only 11 esterase negative patients, of which five had cancer and six other conditions, out of a total of 90 esterase negative patients. Attributing esterase deficiency to an inheritable form, therefore, is not definitely known as the cause in most patients in their study. Their results are certainly important, however, and could lead to future studies which might elucidate these questions.

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Dr Markey comments:

The Haemalog D automated differential white cell counter, producing as it did, a monocyte count on esterase stained cells and a monocyte count on peroxidase stained cells (screening 10 000 cells on each channel) was an excellent system for identifying monocyte esterase deficiency. It is indeed unfortunate that subsequent improvements to its technology involved relinquishing the esterase channel. The semiautomated method of Ross *et al*, however, which involves the manual staining for esterase of whole blood samples and counting the stained cells on the current Technicon H1 system, should permit confirmation of our results. A peroxidase monocyte count by the H1 must also be recorded for each sample because a low esterase percentage count can occur due to many other reasons, whereas only in esterase deficiency will a low recording on the esterase channel be accompanied by a normal count on the peroxidase channel.

We do not believe that the excess of esterase deficient subjects among cancer patients can be due to differences in drug exposure because treatment regimens for different malignant diseases are reasonably standard for each disease group in this hospital and the radiotherapy centre, yet only a small proportion of any malignant disease group manifes-

ted the anomaly. Moreover, of the group of patients with carcinoma, six had not had any chemotherapy or radiotherapy (primary diagnostic beds), while six had (oncology specialist beds). Neither have we any evidence that our subjects' deficiency was acquired as a result of exposure to organophosphates. Levine *et al* (as referred to by Ross) did not report any follow up of their esterase deficient subjects after they had been removed from the plastics production process, but one may reasonably expect recovery of monocyte esterase in newly developing monocytes when organophosphates disappear from the blood stream, as occurred within 14 days of acute organophosphate poisoning in the case reported by Oehmichen *et al*.¹ Fifty of our subjects had confirmatory samples taken over 14 days (mean 17 weeks) following the initial Haemalog D sample and many have had repeat samples since then. None has reverted to positivity. Moreover, we have no evidence of exposure to organophosphates, such as has been reported by Levine *et al*. On the contrary, we have evidence of familial deficiency in nine of 11 families studied (and in one family of three studied in the interim period). At this time we therefore feel that the balance of our evidence is in favour of an inheritable basis for the deficiency in our subjects.

As to our hypothesis that esterase deficiency may predispose to malignant neoplasia, we now have evidence that esterase negative monocytes do not respond to lactoferrin stimulation, with an increase in cytotoxicity for K562 cells, using a modified assay for measurement of monocyte cytotoxicity²; esterase positive monocytes respond vigorously (unpublished observations).

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AgNORs and follicular lymphomas

It was of interest that Cronin and colleagues showed no significant difference between AgNOR scores in follicular hyperplasia and follicular lymphoma.¹ This confirms the results of an original study in this laboratory some three years ago,² where there was no numerical difference in AgNOR score between low grade lymphomas and hyperplastic nodes. The slight trend to higher counts in benign lesions, shown by Cronin *et al*,¹ is to be expected, as in most somatic tissues and neoplasms, and certainly in non-Hodgkin's lymphomas, the interphase AgNOR score has been shown repeatedly to be related to cell proliferation.³ This latter is generally taken to be greater in hyperplastic than malignant (low grade) follicle centres. These observations would presumably only hold true if follicle centre cells are enumerated; unfortunately, this is not made clear by Cronin *et al*. AgNOR scores, however, can discriminate between hyperplastic follicles and high grade (pure centroblastic) follicles. I have examined 10 specimens of reactive follicular hyperplasia and 10 of centroblastic follicular lymphoma using the standard AgNOR sequence² with 3 μ m paraffin wax sections. The latter

had been shown to be of a B cell derivation by means of standard immunophenotyping; eight were detected de novo, and two had arisen by transformation from centroblastic-centrocytic lymphomas. All AgNOR dots, both intra- and extra-nucleolar, were counted in 200 follicle centre cells from each case. The cells were selected at random from all levels of the follicle centres, excluding the mantle zone. The range of values for reactive follicular hyperplasia was from 2.3 to 3.5 (mean 2.85; SD 0.43) AgNOR sites per nuclear profile. In the centroblastic follicular lymphoma specimens the range was from 4.8 to 10.1 (mean 6.12; SD 1.75) (figure). Thus we have yet another example of a malignant transformation carrying with it a higher mean AgNOR score, doubtless as a result of the proliferative overgrowth of neoplastic centroblasts, which themselves have high mean AgNOR counts.

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Dr Cronin *et al* comment:

We thank Dr Crocker for his interest in our small study. Essentially we agree with his comments. The point of our paper was that although we did find a statistical difference between the two groups studied (follicular lymphoma and follicular hyperplasia) the overlap was such that AgNOR counts had no discriminant value as a diagnostic test in the group of patients we examined. As stated in our paper, we confined our study to the centroblastic/centrocytic category of follicular lymphomas; we evaluated cells randomly within follicles, while omitting identifiable macrophages. In our experience (about 80-100 cases of malignant lymphomas a year) pure centroblastic lymphoma is