

Letters to the Editor

Ploidy studies in adenomatous polyps of colon

I read the paper by Whitehead *et al*¹ with great interest as their findings parallel some results, which Dr George Sledge and I have obtained with germ cell neoplasms of the testis.

The conclusion that, "Dukes's (sic) A tumours which have invaded no farther than the bowel wall, if they are aneuploid, exhibit the less severe form (type 3) re-emphasises the importance of early recognition and removal of both adenomatous polyps and carcinomas of the colon" is misleading and not supported by the data in table 1.¹ It is inconsistent to construe that the difference between zero instances of type 4 histograms in stage A carcinomas, two instances in stage B, and one in stage C is meaningful and at the same time draw no conclusion from the observation that 11 of the 14 instances of type 1 histogram occurred in the tumours that had behaved most aggressively. Thus from the data one might better conclude that aneuploidy does not correlate well with neoplastic progression and aggressiveness in colonic epithelial neoplasia. It seems to occur only in malignant neoplasms, but it could be argued that the data would support a concept that it is merely a side effect of the fundamental disorder of growth, and perhaps even one which gets in the way.

The same arguments apply to their conclusions in the last paragraph that, "the findings in this study also support the view that the malignant process in cancer of the colon is a stepwise process." Their conclusion, "There is clearly a case for ploidy studies on all adenocarcinomas of the colon, especially with the technique used in this study, which is simple and cost effective. . . ." is also questionable as the technique entails much special equipment and software not available in most hospital pathology laboratories and as their data do not show a correlation between ploidy and prognosis in colonic adenocarcinoma.

Lastly, although the authors state in the discussion that "25 of the 41 carcinomas examined were diploid" and (referring to textual citations of 15 cases of type 1 histogram, 10 cases of type 2, 13 cases of type 3, and 3 cases of type 4) that "Tables 1 and 2 summarise these results," table 1 tabulates only 36 carcinomas altogether and shows only 24 of them as diploid. The two tables also show 27 instances of type 1 histogram (14 carcinomas, 13 adenomas), 13 instances

of type 2 (10 carcinomas, 3 adenomas), nine instances of type 3 (all carcinoma), and three instances of type 4 (all carcinoma). Thus the text and the tables disagree on the number of carcinomas, the number of diploid carcinomas, and the numbers of types 1 and 2 cases.

JOHN N EBLE
Chief, Laboratory Service,
Roudebush Veterans Administration Center,
1481 West 10th Street,
Indianapolis IN 46202,
USA

Reference

- 1 Whitehead F, Jarvis LR, Skinner JM, Whitehead R. Ploidy studies in adenomatous polyps of the colon. *J Clin Pathol* 1985; 38:1261-4.

Dr Whitehead and colleagues reply:

We are pleased that Dr Eble has found our paper to be of such interest, and we appreciate his detailed appraisal. We are surprised, however, by the comment that we drew no conclusion from the fact that most of the invasive carcinomas showed an apparently diploid characteristic. Our discussion makes clear reference to this observation, and we conclude that in the cases we examined abnormality in ploidy distribution does not seem to correlate well with histological features of malignancy. We are in complete agreement with Dr Eble's comments on this point. As to ploidy abnormality being a "side effect" and a feature of disordered growth that "gets in the way" (presumably of invasion), we cannot argue that our results support such a hypothesis. We have suggested that the results indicate that the change from diploid to aneuploid may occur after the disorder that determines the property of invasiveness and thereby represent a new adverse trend in those tumours and that this supports the notion of a stepwise malignant process with increasing epithelial abnormality. This concept has been admirably discussed and supported by ploidy analysis of a large number of adenomas and carcinomas, in a recent publication in this journal.¹

Whatever conclusion is drawn from our work, it remains clear from other studies that ploidy abnormality is a feature of adenocarcinoma of the colon that correlates well with poor prognosis, and furthermore, recent studies have shown that aneuploidy occurs in adenomas.¹ From such published evidence there is, indeed, a case for ploidy analysis on all adenocarcinomas of the

colon. Our suggestion is that the techniques used in our study, being based on microcomputer technology, make the analysis cost effective. Although the necessary equipment will not be available in most hospital laboratories, we would be pleased to assist any interested group in establishing such a facility.

F WHITEHEAD
LR JARVIS
JM SKINNER
R WHITEHEAD
Department of Pathology,
Flinders Medical Centre,
Bedford Park, 5042,
South Australia

Reference

- 1 Goh HS, Jass JR. DNA content and adenoma-carcinoma sequence in the colon and rectum. *J Clin Pathol* 1986;39:387-92.

False positive results with an ELISA for detection of chlamydia antigen

A recent editorial in the *Lancet*¹ highlighted the growing recognition of the morbidity associated with *Chlamydia trachomatis* in women. It also posed the question of whether adequate diagnostic facilities could be made available to screen groups at risk.

Apart from their cost, enzyme immunoassays have the potential to be used for screening large numbers of specimens. Pugh *et al* recently reported favourably on an enzyme amplified immunoassay for diagnosis of *C trachomatis*.² We performed a small evaluative study of our own, comparing the same immunoassay system with a conventional chlamydia culture system. During the study, six routinely submitted rectal swabs were examined, and to our surprise four of the six gave strong positive results on the enzyme immunoassay. These specimens were all negative by culture, as were the corresponding urethral swabs. We suspected that the enzyme immunoassay results probably represented false positives and therefore investigated this problem further.

All swabs were received in our conventional chlamydia transport medium. Prior evaluation had shown this to give satisfactory results in the enzyme immunoassay with genital specimens. It had the advantage that the same swab could be tested by both culture and enzyme immunoassay. Culture was performed by inoculating specimens to coverslips of McCoy cells treated with cycloheximide. Each specimen was inoculated

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Table 1 Comparison of chlamydia culture and enzyme immunoassay using rectal swabs

Enzyme immunoassay	Culture			
	Positive	Negative	Contaminated	Total
Positive	1	16	1	18
Equivocal	0	1	0	1
Negative	0	2	2	4
Total	1	19	3	23

lated on to two coverslips. After 48 hours incubation coverslips were stained with Giemsa and examined for inclusions. Enzyme immunoassay was performed according to the manufacturer's instructions (IDEIA, Boot's Celltech).

A total of 23 rectal swabs have now been examined. As can be seen in the table, 17 of 23 gave clear positives with the enzyme immunoassay, but only one of these specimens was positive by culture. Urethral swabs were received in parallel with the rectal swabs, which gave discrepant results, and all 16 gave negative culture results. It was clear from these results that the enzyme immunoassay was producing false positive results with rectal swabs. This conclusion was supported by the finding that 15 of 16 swabs taken from randomly selected faeces submitted to the Public Health Laboratory also gave strong positive results in the enzyme immunoassay.

In view of the very strong positive results obtained with faeces and rectal swabs a bacterial antigen cross reacting with the anti-chlamydial monoclonal antibody seemed unlikely. The organisms listed in table 2, however, were tested in the enzyme immunoassay by making heavy suspensions in chlamydia transport medium. All gave negative results in the enzyme immunoassay, including the strain of *Acinetobacter calcoaceticus* var *anitratus*. This organism has been reported³ to give positive results with another enzyme immunoassay for chlamydia (Chlamydiazyme, Abbot Laboratories).

We proceeded to investigate whether protein A of staphylococci could reproduce the effect. Suspensions of six strains were tested.

Four of the five *Staphylococcus aureus* strains gave positive results, whereas the *S epidermidis* strain was negative. Positive results were also produced by a preparation of protein A. It seems unlikely, however, to be the explanation for false positives with faeces and rectal swabs, as when serial dilutions of *S aureus* were tested it was established that an inoculum of 10^3 organisms/ml was required to produce positive results. This is clearly far larger than the likely numbers of *S aureus* in either faeces or rectal swabs.

We have therefore been unable to identify the precise mechanism of the false positive results, but it would seem likely that it entails a non-specific binding to mouse immunoglobulin, as in our studies prior absorption of faeces with mouse serum gave a two fold reduction in reading in the enzyme immunoassays, compared with absorption in transport medium.

We have shown that this kit is quite unsuitable for examining rectal swabs, although in fairness the manufacturer's instructions indicate that it is designed for examining genital specimens only. We consider, however, that this problem should be investigated further to ensure that it cannot produce occasional false positive results, with genital specimens. Even if false positive results were uncommon, they would assume importance if the kit were used to screen populations, such as antenatal clinics, which may have a low prevalence of true positive results, rather than the high incidence in the populations examined in reported studies.

T RIORDAN

DA ELLIS

PI MATTHEWS

SF RATCLIFFE

Central Serology Laboratory,
Withington Hospital,
West Didsbury,
Manchester M20 8LR

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- 2 Pugh SF, Slack RCB, Caul EO, Paul ID, Appleton PN, Gatley S. Enzyme amplified immunoassay: a novel technique applied to direct detection of Chlamydia trachomatis in clinical specimens. *J Clin Pathol* 1985;38: 1139-41.
- 3 Saikku P, Puolakkainen M, Nissinen A. Cross-reactivity between chlamydiazyme and Acinetobacter strains. *N Engl J Med* 1986;314:922.

Human parvovirus associated with erythroblastopenia in iron deficiency anaemia

Anderson *et al*¹ have shown that human parvovirus (HPV) is responsible for aplastic crises in patients with chronic haemolytic anaemia. We report an HPV-aplastic crisis, with rapidly worsening anaemia in a patient without an underlying haemolytic anaemia.

A 28 year old woman presented with a seven day history of abdominal pain, headache, and fever (39°C). She had had iron deficiency anaemia (haemoglobin concentration = 10 g/dl, mean cell volume = 56 fl) for many months and showed non-compliance with iron treatment. Examination showed that there were no signs, apart from pallor and an ejection systolic murmur. Liver, spleen, and lymph nodes were not enlarged. Her haemoglobin concentration was 7 g/dl, her mean cell volume 69 fl, reticulocyte count $5 \times 10^9/l$, white cell count $1.5 \times 10^9/l$, and platelet count $130 \times 10^9/l$. Her bone marrow was hypocellular, with erythroid aplasia (1%), absence of megakaryocytes, normal granulocyte maturation, and 2% giant cells (diameter $100 \mu m^3$) with blue cytoplasm. Her total bilirubin concentration was $10 \mu mol/l$ and serum iron $7 \mu mol/l$. Her serum was negative for HPV antigen, but recent HPV infection was suggested by the presence of specific IgM in radioimmunoassay. Other infections excluded were hepatitis A and B, infectious mononucleosis, and cytomegalovirus. A search for an underlying haemolytic anaemia (blood film, study of osmotic resistance, in vitro autohaemolysis, haemoglobin electrophoresis, erythrocyte enzymatic tests, direct Coombs' test) was negative. She was given three units of packed red cells, after which her haemoglobin rose to 11.3 g/dl. The fever disappeared within two days, and the thrombocytopenia and neutropenia spontaneously resolved with five days by which time the reticulocyte count was high ($250 \times 10^9/l$). A second bone marrow smear showed normoblastic erythroid hyperplasia (34%), the presence of megakaryocytes, and the disappearance of the giant cells seen on the first bone marrow smear.

Table 2 List of faecal organisms tested by enzyme immunoassays

<i>Acinetobacter calcoaceticus</i> var <i>anitratus</i>
<i>Bacteroides fragilis</i>
<i>Bacteroides melaninogenicus</i>
<i>Bacteroides ovatus</i>
<i>Escherichia coli</i>
<i>Fusobacterium fusiforme</i>
<i>Klebsiella aerogenes</i>
<i>Salmonella typhimurium</i>