

DNA aneuploidy in sarcoid granuloma

Nuclear DNA aneuploidy measured by flow cytometry on fresh or paraffin wax embedded tissue has been reported in several malignant diseases.¹ DNA aneuploidy has also been shown to occur in histologically benign tumours, such as cutaneous naevi and adenomas of endocrine glands and the colon.¹ Reports of aneuploidy in non-neoplastic, proliferating states have primarily concerned lesions that are known to be premalignant, such as ulcerative colitis² and Barrett's esophagus.³

We analysed the distribution of nuclear DNA content by flow cytometry on paraffin wax embedded tissue from 10 Boeck's sarcoid lesions, five non-specific inflammatory granulomas, and five granulomas associated with silicon breast prostheses. Paraffin wax sections (40 µm) were rehydrated, trypsinated overnight, and stained with propidium iodide, according to a modification of the method of Schutte *et al.*⁴

In two of the cases, a 50 year old woman with a Boeck's sarcoid lesion in a renal biopsy specimen (fig 1a) and a 34 year old woman with a granulomatous lesion associated with a tubo-ovarian abscess (fig 1b), the DNA fluorescence histograms showed a well separated secondary peak. The secondary peak had a DNA index of 1.3 and 1.1 and represented between 30% and 45% of the nuclei, respectively. An additional case, a 42 year old woman with a

granuloma in relation to a ruptured silicon breast prosthesis, showed a right skewed peak, similar to that found in DNA histograms of cutaneous lymphoid infiltrates.⁵

Control specimens from normal breast tissue had a single symmetrical peak with coefficient of variation (3.9–5.6%). Follow up on the three patients was in excess of three and a half years and none showed signs of neoplastic disease. The patient with Boeck's sarcoid had been receiving long term, low dose (7½ mg daily) prednisone treatment, and the patient with the tubo-ovarian abscess had been on a combination low dose contraceptive pill until recently when a tubal ligation was performed. No medication had otherwise been prescribed.

The finding of pronounced DNA aneuploidy in histologically benign lesions from Boeck's sarcoid and granulation tissue is interesting. If the findings represent a malignant process, steroid treatment and the surgical intervention may have been curative. Furthermore, with an observation period of three and a half years, the possibility of a slowly growing malignancy cannot be entirely excluded. On the other hand, as the abnormal DNA content was found in one of the 10 patients with Boeck's sarcoid and in one of five patients with non-specific granulomas, this may have been an epiphenomenon in lesions with pathological, but non-neoplastic proliferation. The multinucleate giant cells present in both lesions could be a source of the aneuploidy.

If so, some change in the DNA would seem necessary as mere agglutination of giant cell nuclei during the analysis would yield DNA indices higher than the observed values in the hypotriploid range. Other cells in the granulomatous lesions (fibroblasts, macrophages) could also be implicated as possible sources for the DNA aneuploidy. Clearly, multiparametric flow cytometric analysis on fresh tissue would be needed to pinpoint further the abnormal cell population(s).

The finding of DNA aneuploidy by flow cytometry in lesions with no malignant potential suggests that DNA aneuploidy in the absence of clinical and histological evidence of malignancy should be interpreted with caution.

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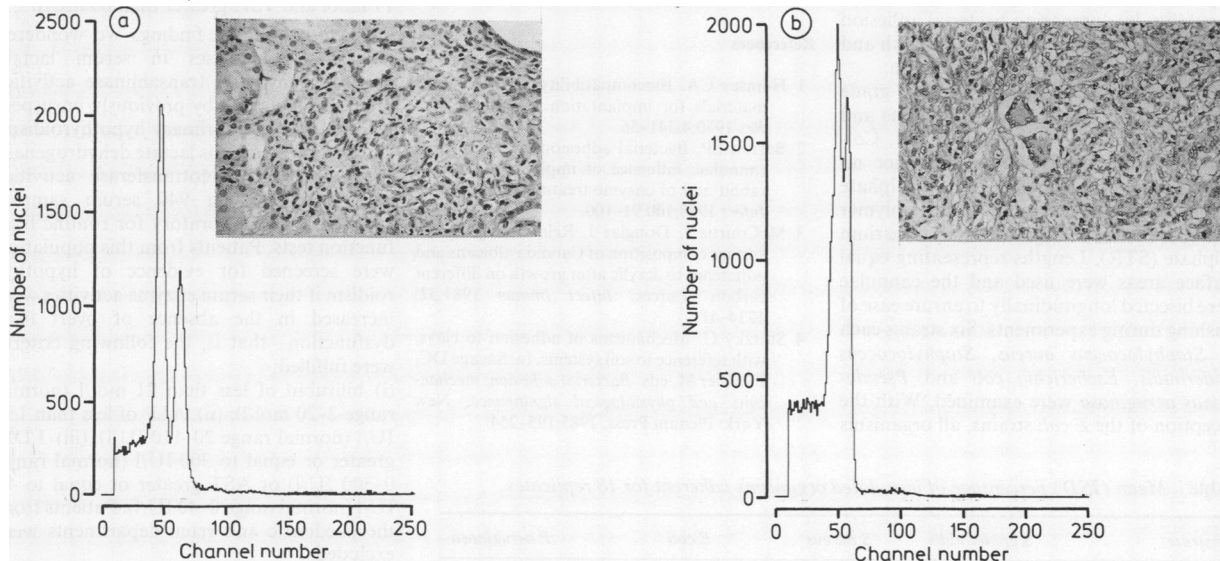


Figure DNA fluorescence histograms from two cases of granulation tissue. Numbers of nuclei counted are on the ordinate and 256 channels of fluorescence intensity are on the abscissa. Histograms from Boeck's sarcoid lesion (a) and granulation tissue associated with tubo-ovarian abscess (b) both show well separated secondary peaks. Inset: microscopic appearance of the analysed tissue specimens.

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Barium sulphate radio-opacity agent and bacterial adhesion to silicone catheter material

Intravascular cannulae are often impregnated with barium salts to render them radio-opaque. The biocompatibility¹ of silicone based polymers makes them a natural choice in the manufacture of longer term intravascular devices, and silicone cannulae impregnated with barium display a greater affinity for bacteria than do other implanted plastics.² As divalent cations have a recognised role in the adhesion of micro-organisms to inanimate surfaces^{3,4} it is theoretically possible that cannula material impregnated with barium might be more prone to bacterial colonisation and subsequent infection. We investigated this possibility by comparing bacterial adhesion to a silicone cannula material both with and without barium sulphate impregnation.

Dow Corning silicone rubber, grade RX65, was obtained as central venous and drainage cannulae (HG Wallace Ltd, Colchester, England). It contained either no barium sulphate (RX), 8% barium sulphate evenly distributed throughout the polymer (BaS), or a narrow lengthwise strip of barium sulphate (STR). Lengths representing equal surface areas were used and the cannulae were bisected longitudinally to ensure ease of washing during experiments. Six strains each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* were examined. With the exception of the *E coli* strains, all organisms

had been obtained from infected intravascular cannulae. The bacteria were grown overnight on nutrient agar containing ³H-glucose and washed three times by centrifugation before use. Each strain was suspended in dulbecco AB buffer and 1 ml was placed in a stoppered container along with one of the cannula materials. The containers were tumbled for one hour at room temperature to allow adhesion to take place. At the end of the experiment loosely adherent bacteria were removed by repeated washing in buffer. All experiments were performed in triplicate. When dry, the pieces of cannula were immersed in scintillation fluid and β emissions were counted. The percentage of counts adherent to cannula material are shown in the table.

Comparison of the variance of adhesion of bacteria (F-test) showed no significant differences in adhesion to the three preparations of the polymer.

The above findings indicate that the presence of barium sulphate does not influence the adhesion of bacteria to silicone polymer and that its use as a radio-opacity agent is unlikely to influence bacterial colonisation of intravascular cannulae.

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Hypothyroidism: an important differential diagnosis for raised serum transaminases?

Raised serum transaminase and lactate dehydrogenase activities are often found in the absence of any overt liver dysfunction. When assessing such abnormalities, it is widely believed that hypothyroidism must be considered as this is known to be associated with increases in several serum enzymes including lactate dehydrogenase (LDH), creatine kinase, aldolase, transaminases, malate dehydrogenase and hydroxybutyrate dehydrogenase.^{1,2} This association can be of diagnostic importance as clinicians have, on occasion, wrongly attributed increased activities of "cardiac enzymes" in patients with primary hypothyroidism to ischaemic heart disease.^{3,4}

Two cases from Torbay Hospital were brought to our attention (Yates, personal communication) in whom asymptomatic primary hypothyroidism was diagnosed following the further investigation of abnormal enzymes on routine blood testing. Both were men aged 61 and 31, respectively, with normal serum bilirubin concentrations and alkaline phosphatase (ALP) activities. Serum aspartate amino transferase (AST), LDH, thyroxine (T4), and thyroid stimulating hormone (TSH), however, were abnormal as shown below. In case 1 AST was 488 U/l (5-40), LDH 717 U/l (150-450), T4 64 nmol (60-160) and TSH 14 mU/l (up to 8). In case 2 AST was 66 U/l, LDH 1057 U/l, T4 17 nmol and TSH greater than 60 mU/l.

Because of these findings we wondered how often increases in serum lactate dehydrogenase and transaminase activities might be explained by previously unsuspected or developing primary hypothyroidism. To test the hypothesis lactate dehydrogenase and aspartate aminotransferase activities were measured on 940 serum samples received by the laboratory for routine liver function tests. Patients from this population were screened for evidence of hypothyroidism if their serum enzyme activities were increased in the absence of overt liver dysfunction—that is, the following criteria were fulfilled:

(i) bilirubin of less than 21 mol/l (normal range 2-20 mol/l); (ii) ALP of less than 150 IU/l (normal range 20-110 IU/l); (iii) LDH greater or equal to 300 IU/l (normal range 0-300 IU/l) or AST greater or equal to 40 IU/l (normal range 0-40 IU/l). Patients from the paediatric and renal departments were excluded.

One hundred and twenty five samples from 62 women and 63 men fulfilled the defined criteria. LDH activities alone were

Table Mean (SD) percentage of inoculated organisms adherent for 18 replicates

Substrate	<i>S epidermidis</i>	<i>S aureus</i>	<i>E coli</i>	<i>P aeruginosa</i>
Silicone (BaS)	16.7 (12.0)	22.6 (25.0)	4.0 (2.7)	50.8 (24.0)
Silicone (STR)	13.5 (11.3)	25.1 (23.5)	4.5 (2.2)	46.9 (24.0)
Silicone (RX)	21.2 (12.0)	36.0 (29.0)	5.2 (3.4)	50.4 (29.0)