Positive reactions seen at each site (percentages in parentheses)

Tissue	Pancreatic polypeptide	EMA	PGP9·5	Serotonin	Grimelius
Small intestine	0 (0)	0 (0)	4 (75)	4 (75)	6 (100)
Pancreas	2 (29)	1 (14)	6 (86)	2 (29)	3 (43)
Lung	0 (0)	1 (50)	2 (100)	1 (50)	0 (0)
Stomach	1 (33)	2 (66)	1 (33)	0 (0)	2 (66)
Rectum	2 (40)	2 (40)	3 (60)	2 (40)	0 (0)
Appendix	0 (0)	1 (17)	4 (67)	6 (100)	6 (100)

Carcinoid tumours from various sites were withdrawn from the routine surgical pathology files of the General Infirmary at Leeds. These comprised six from the small intestine, two from the lung, three from the stomach, five from the rectum, six from the appendix, and seven islet cell tumours of the pancreas. Only those tumours were included in which the diagnosis was confirmed by either positive silver stains or demonstration of dense core neurosecretory granules by electron microscopical examination. All material was formalin fixed and paraffin wax embedded. One representative block from each tumour was selected.

Serial sections from each tumour were stained with haematoxylin and eosin and impregnated with silver by the Grimelius technique. Four immunohistochemical reagents were applied: PGP 9·5 (UltraClone Ltd, dilution 1 in 400), anti-human pancreatic polypeptide (Dakopatts, prediluted), serotonin (Seralab, dilution 1 in 100) and monoclonal anti-epithelial membrane antibody (Dako, dilution 1 in 10). Pretreatment with trypsin was used for all except PGP 9·5.[1] The predominant histological pattern of each tumour was determined according to Dawson's classification,[2] and positive or negative staining with each reagent noted. Focal staining was regarded as positive provided that it was of sufficient intensity and clearly localised to tumour cells.

The results are shown in the table. A small proportion of carcinoid tumours give a clear positive reaction to epithelial membrane antigen (EMA). The most common pattern was a diffuse, granular cytoplasmic staining, but apical membrane staining was also seen around lumina. Positive staining showed no correlation with histological subtype, nor with site.

EMA is an antibody to a specific component of the human milk fat globule membrane.[3] It has been shown to react with a wide variety of benign and neoplastic epithelia, and has proved a useful tool in the categorisation of undifferentiated tumours. Experience, however, has shown a more widespread distribution than was initially thought. Previous studies included very few carcinoid tumours. These were reported as unreactive[4,5] or as showing isolated positive staining.[6,7]

It is unwise to draw conclusions about cell of origin of a neoplasm from expression of antigen, but it is sometimes possible to predict antigen expression if the cell line is known. Carcinoid tumours are believed to originate from the mucosal endocrine cell. It is suggested that these in turn derive from a common endodermal stem cell. As most endoderm expresses EMA, occasional expression of EMA by carcinoid tumours comes as no surprise. It is important to be aware of this possibility, as otherwise the unexpected finding of a positive reaction to EMA with a dubious or negative silver stain may mislead the unwary.

A GLEDHILL
C SPARHAM
MF DIXON
Department of Pathology, University of Leeds, Leeds LS2 9JT

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MATTERS ARISING

Nucleolar organiser regions in renal tumours

Bryan, Crocker, and Farr recently compared nucleolar organiser region (NOR) numbers in seven cases of renal "adenoma", nine cases of renal cell carcinoma, and five cases of xanthogranulomatous pyelonephritis.[1] Using the standard silver colloid staining technique they showed a significant difference between mean NOR values for renal cell carcinoma and xanthogranulomatous pyelonephritis but not between renal cell carcinoma and renal "adenomas". While not disputing the observations of these authors, we are of the opinion that their report does not provide an accurate indication as to the range of NORs seen in renal cell carcinoma.

Delahunt *et al* recently reported on NOR numbers in 75 cases of renal cell carcinoma, eight renal oncocytomas, and nine renal "adenomas".[2] They graded the renal cell carcinomas and found a significant difference in mean NOR numbers between each of the assigned grades of renal cell carcinoma and between renal "adenomas" and oncocytomas when compared with grades II and III renal cell carcinomas. There was no difference in mean numbers of NORs of oncocytomas, "adenomas", and grade I renal cell carcinomas.

There is increasing evidence to suggest that the number of detectable NORs within nuclei is a reflection of the proliferative activity of the tissue examined.[3,4] It has been further shown that higher grades of malignant tumours have greater detectable numbers of NORs, using the silver colloid stain, than similar tumours of lower grade.[5,6]

Bryan *et al* did not grade the malignant tumours in their study, and the only indication they provide as to the grade of the renal cell carcinomas in their series is given in an illustration. This shows a low grade clear cell renal cell carcinoma which would be expected to have a mean NOR value within the range found in renal "adenomas".

The report of Bryan *et al* serves to emphasise that when NOR counts from different tumours are compared, the grade of the tumour should be taken into account and if this is not done then incorrect conclusions may be derived.

B DELAHUNT
WJ FRATER
Department of Pathology
JN NACEY
Department of Surgery, Wellington School of Medicine, Wellington, New Zealand

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Drs Bryan and Crocker comment:

We were interested to observe the comments of Delahunt *et al* from New Zealand regarding our study of AgNORs in renal neoplasms. We must make one or two points which will clarify the questions they have raised.

Firstly, we applied the AgNOR technique to renal cell carcinoma, adenoma, and xanthogranulomatous pyelonephritis *in an attempt to facilitate differentiation.* The continuous range in AgNOR counts shown by us in renal neoplasms suggested that this supported the notion of a continuous range of renal tumours. We therefore pointed out that the AgNOR technique is not able to distinguish tumours expected to behave in a benign manner from those expected to have a malignant natural history. The results of Delahunt *et al* are similar, with no significant difference in mean AgNOR counts for "adenoma" and grade 1 renal cell carcinoma. These observations would also support the hypothesis of a continuous spectrum of renal tumours. Naturally it is to be expected that high grade renal cell carcinoma would have a higher AgNOR score than low grade tumours, but conventional light microscopical examination

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should be sufficient to differentiate the former from renal adenoma.

Secondly, our study was small and not intended to provide an accurate indication of the range of NOR numbers seen in renal cell carcinoma. As indicated above, our remit was quite different from that of Delahunt *et al.* Indeed, our figures show that there is a large range of NOR counts in renal carcinoma. We suggested that further study would be necessary before firm conclusions could be drawn.

Thirdly, we are not quite sure of the meaning of the authors' final sentence ". . . . if this is not done then incorrect conclusions may be derived". This is a perplexing statement because Delahunt *et al* did not seem to disagree with our observations.

Finally, we should like to indicate that our paper was written and accepted a long time before publication as a result of technical problems beyond our control. Had the paper been written more recently we should, of course, have been aware of, and acknowledged, the study by Delahunt *et al.*

Cellularity and oestrogen receptor content in breast cancer microsamples

In a recent issue of the Journal Parham *et al* evaluated the relation between breast cancer cellularity and oestrogen receptor (ER) content.[1] The authors concluded that, "there is no single direct correlation between tumour cellularity and oestrogen receptor content". We feel that such a definitive conclusion cannot be drawn from this study, primarily because of its sampling technique. It is well established that the ER content of a tumour exhibits regional variability,[2,3] thus to assess the effect of cellularity on receptor content, it is important that these variables are assayed using immediately adjacent samples. In Parham's study the cellularity was assessed retrospectively using tissue sections which are assumed to represent the tumour as a whole, and the portion submitted for ER analysis in particular.

We suggest that this sampling procedure does not address the issue of regional variability and therefore agree with the authors' statement that, "it may be possible to obtain a better correlation if contiguous samples are taken". We have addressed the issue of cellularity, and its effect on ER content using a "micromethod" technique.[4] This procedure attempts to correct for regional variability by assaying contiguous samples. Briefly, a 40 mg "microsample" is divided in half lengthwise so that one half can be analysed for cellularity and the other for ER. Using this method, the two analyses are performed on tissue samples that are no more than 1 mm apart.

This technique was used to investigate the possibility that fluctuations in ER content are not random, but are associated with specific regions of a tumour (peripheral, intermediate, central). The ER content reported in that study[5] was a composite of biochemical and histological data, but the issue of cellularity and ER content was not addressed directly. Using these data, we calculated the correlation between cellularity and ER content in 25 breast tumours. The results are summarised in the table. It should be noted that a total of five samples were taken from each tumour, size permitting. We acknowledge that the assessment of cellularity is a potential source of error and have attempted to minimise this problem by using the estimates of four independent observers. In a recent study these estimates fell within 10% of the mean (n = 107).[5]

Our data show that the correlation between cellularity and ER is highly variable, both between and within tumours. This observation indicates that the authors' assumption that ER content and cellularity can be evaluated at different regions may not necessarily be correct. Experimental evidence thus corroborates our argument that cellularity and ER measurement should be made on contiguous samples. In our study the overall correlation between cellularity and ER, for ER positive tumours was r = 0·68 (n = 20); for ER positive as well as negative tumours the correlation was much lower, r = 0·33 (n = 25) but still positive. We believe, however, that ER negative tumours should be excluded from the analysis as, clearly, ER content and cellularity are not related. In conclusion, we believe that it is premature for the authors to conclude that there is "no single direct correlation between tumour cellularity and ER content" in breast cancer.

JP VAN NETTEN
Department of Pathology, Royal Jubilee Hospital, 1900 Fort Street, Victoria, British Columbia, Canada V8R 1J8
P COY
Victoria Cancer Clinic, Cancer Control Agency of British Columbia
C FLETCHER
Special Development Laboratory, Great Victoria Hospital Society

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Dr DM Parham et al comment

The design of our study did not allow contiguous tissue samples to be examined. We believe, however, that this does not invalidate our findings or conclusions.

A previous study by van Netten *et al* in 1988 using the "micromethod technique" would suggest that regional variation in oestrogen receptor content may not be important. They showed that the average oestrogen receptor content, when corrected for cellularity, was 20% higher in the intermediate zone than in the tumour centre or periphery. The mean oestrogen receptor contents in the different regions of 25 tumours corrected for cellularity were: central zone

Comparison between cellularity (%) and oestrogen receptor content in breast cancers

Patient's age	*Region within a tumour*										*Intratumour Correlation (r) Cellularity v ER*
	Peripheral				*Intermediate*				*Central*		
	Cellularity	*ER*	*Cellularity*	*ER*	*Cellularity*	*ER*	*Cellularity*	*ER*	*Cellularity*	*ER*	
49	18	3	16	18	19	4	8	*	25	9	− 0·25 (n = 4)
53	6	3	3		78	105	36	192	95	155	+ 0·54 (n = 4)
61	83	0	60	0	68	0	60		25	0	0·00 (n = 4)
61	80	75	85	191	75	174	78	193	88	177	+ 0·14 (n = 5)
63	20	6	16	15	14	16	20	15	14	18	− 0·71 (n = 5)
65	33	36			30	39			26	75	− 0·93 (n = 3)
66	20	0	3		18	0	17	0	20	0	0·00 (n = 4)
67	13	82			7	39			5	9	+ 0·98 (n = 3)
67	7				13	5			9	3	+ 1.00 (n = 2)
70	29	55	19	47	34	71	21	56	25	39	+ 0·67 (n = 5)
73	5	0	5	0	13	0			13	0	0·00 (n = 4)
73	5	0	70	16	15	0	8	0			+ 0·00 (n = 4)
73	9	0	80	0	71	0	71	0	50	0	0·00 (n = 5)
74	56	86	90	149	80	29	88	83	90	83	+ 0·23 (n = 5)
76	25	24	6	30	36	69	20	58	33	78	+ 0·69 (n = 5)
77	15	34	3		8	25	16	53	14	36	+ 0·80 (n = 4)
77	19	37	23	24	7	21					+ 0·44 (n = 3)
78	80	101	53	84	66	90	54	80	39	70	+ 0·99 (n = 5)
79	41	27			56	16			71	84	+ 0·78 (n = 3)
79	28	6			18	13			10	8	− 0·34 (n = 3)
81	70	23	16	14	38	75	35	69	41	50	− 0·04 (n = 5)
85	19	89	5	33	23	57	10	106	11	73	+ 0·20 (n = 5)
87	34	61	13	95	14	75	34	102	25	64	− 0·13 (n = 5)
89	100	0	100	0	100	0	100	0	100	0	0·00 (n = 5)
94	35	36	70	49	83	51	28	41	65	36	+ 0·66 (n = 5)

*Sample not analysed.

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