# A COMPARISON OF THE DNA CONTENT AND CHROMOSOME NUMBER OF FIFTY HUMAN TUMOURS

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THE preparation of tumour material for the measurement of the stain content of individual cells is a relatively simple procedure, and the technique of Feulgen microspectrophotometry can be applied to virtually all samples of fresh tumour material ; indeed, adequate data on deoxyribonucleic acid (DNA) content can thereby be obtained not only from pieces of solid tumour removed surgically, but also from smears consisting only of moderate numbers of interphase cells, obtained for instance by scraping the surface of malignant lesions of the cervix uteri (Atkin, 1964*b*). In contrast, chromosome studies demand material containing adequate numbers of dividing cells (not always present in small samples of human tumour material) and furthermore are subject to the vagaries of present-day techniques for chromosome spreading : metaphases may be poorly spread or have lost chromosomes during the course of preparation.

The object of the present study is to investigate the relation between the DNA content (of *interphase* cells, estimated by microspectrophotometry) and the chromosome number of a fairly large group of human tumours. We have undertaken this investigation for two reasons : to provide more confidence in the use of Feulgen microspectrophotometry of interphase cells for the estimation of the approximate chromosome number of a tumour, and to study in more detail the small discrepancy between DNA content and chromosome number, usually in the direction of more DNA per chromosome than in normal cells, previously observed in a group of 14 tumours (Richards and Atkin, 1960).

Fifty tumours, divided into two groups, have been studied. The main group of 30 tumours comprises those which over the past $2\frac{1}{2}$ years have yielded the most abundant well-spread metaphases ; some of the tumours were suitable for karyotype analysis as well as for chromosome counts, and the DNA data will be considered in relation to the observed karyotype abnormalities. The second group comprises tumours on which only a few exact chromosome counts, or mainly inexact counts, have been obtained. In part, the tumours in this group have been selected on the basis of their DNA content : since the first group contains only a few tumours with high DNA modes, several such tumours have been included in the second group (recent improvements in techniques for chromosome analysis have facilitated this, although a sparsity of metaphases may remain a difficulty).

## MATERIALS AND METHODS

To avoid possible bias, DNA estimations and chromosome counts were made independently, by N. B. A. and G. M., and by M. C. B. respectively. The DNA estimations were made on Feulgen-stained smears prepared from fresh tumour

tissue, or ascitic fluid containing malignant cells, as previously described (Atkin and Richards, 1956). A few of the chromosome counts were made on squash preparations after pretreatment in 1% " Versene ", but the majority were made on air-dried preparations after incubation of the tumour material in the presence of " Colcemid " and in a hypotonic medium (Atkin and Baker, 1966).

*Estimation of the basic DNA value*

Previous findings with the present technique were confirmed : when the DNA contents of a relatively small random sample of say 30 interphase tumour cells are measured, it is nearly always found that the majority are grouped together, although the level of the mode varies widely in different tumours. A few cells may be expected to be synthesising DNA and to have values up to twice the " resting " value for this reason, but clearly these usually form only a minority ; sometimes, there are prominent secondary modes consisting of cells with two and perhaps four times the basic value, presumably a consequence of a high incidence of polyploidization of the basic cell-line. In nearly all the tumours in this study, 30 interphase cell measurements were regarded as sufficient to establish a modal value (although in fact more were usually measured). 20 or more of these cells being within $\pm 15\%$ of a mode. Representative histograms of the DNA contents of random samples of interphase cells from human tumours, obtained with the present technique, have appeared in previous publications (Atkin and Richards, 1956 ; Atkin, 1960, 1962, 1964a, 1964b).

The basic DNA value (Atkin, Richards and Ross, 1959) was calculated as follows. Individual measurements were first plotted as a frequency distribution. The mean stain content, in arbitrary units, of 20 or more (on the average, 30·3) interphase cells falling within $\pm 15\%$ of the mode was then calculated, as was the mean stain content of 15 to 20 leucocytes or fibroblasts, which served as controls. The basic DNA value is the ratio of these two means (tumour/control) $\times$ 100.

From the basic DNA value, an " expected " chromosome number (assuming the same mean DNA content per chromosome as for normal cells) was calculated. In this calculation, allowance has been made for the apparent difference in DNA content between normal epithelial cells and cells of mesenchymal origin : using the present microspectrophotometric technique, uterine epithelial cells were found to have about 10% more stain content than leucocytes or fibroblasts (Atkin and Richards, 1956). In order to define this difference more closely, data from 3 specimens of non-malignant uterine cervical epithelium and 2 of endometrium have been summated (histograms of DNA values from all except one have been previously published : Atkin and Richards, 1956, Fig. 3-6 ; the other specimen is of normal cervix from a patient aged 41 who underwent a hysterectomy following a tubal abortion). The ratio of the mean stain content of 210 epithelial cells to that of approximately the same number of leucocytes and fibroblasts was 1·0987 (standard deviation $\pm 0·0378$). In calculating the standard deviation, the following procedure was adopted : the ratio of the mean stain content of 10 epithelial cells to that of 7-10 leucocytes or fibroblasts situated near to them in the preparation was first calculated, and the final ratio with its standard deviation was calculated from a series of such ratios ; allowance was thereby made for possible regional variation in intensity of staining. It appears therefore that the difference between uterine epithelium on the one hand and leucocytes

and fibroblasts on the other is very close to 10%. A similar difference has been found for other types of epithelium, and for epithelium in the growing as well as the resting phase (unpublished data). Thus, the ratio of the mean stain content of 30 epithelial cells which were within $\pm 15\%$ of the main mode (ignoring cells with higher values which were probably synthesising DNA), derived from endometrium showing benign hyperplasia with frequent mitoses, to that of 30 leucocytes was 1·089 (standard deviation $\pm 0·010$).

We have therefore applied a correction factor of 1·1 in calculating the expected chromosome number from the DNA value where the tumours are of epithelial origin. This factor has also been applied for the two seminomas, although we are uncertain whether a similar difference applies to the homologous normal cells. It has been applied for the teratoma, since the data on this tumour appertain to the carcinomatous elements. Thus, for epithelial tumours:

$$\text{expected chromosome number} = \frac{\text{basic DNA value}}{100} \times \frac{46}{1·1}.$$

For the reticulum cell sarcoma, however, the factor 1·1 was omitted, on the assumption that the homologous normal cells have the same mean stain content as the control cells.

The ratio of the DNA content, in terms of " expected chromosome number ", to the observed chromosome number (conveniently called the *DNA/chromosome ratio*) provides a measure of the average DNA content per chromosome.

## RESULTS

### A. *Main Group of* 30 *Tumours*

The chromosome counts obtained from each tumour are plotted as histograms in Fig. 1. A few counts fell outside the ranges shown in the histograms. Low counts which probably represented broken metaphases were occasionally encountered. There were usually one or two metaphases with about double the modal value, but only in Tumour No. 3 did these form a significant proportion of the total (the high counts have been included in the histogram), and it was noted that in this tumour there was also a correspondingly prominent secondary mode in the DNA distribution. On the whole, the distributions of chromosome counts are fairly symmetrical, and the mean and modal chromosome numbers usually coincide. There is generally no excess of hypomodal over hypermodal counts such as might be expected if there were a significant number of incomplete metaphases which had lost a few chromosomes during the course of preparation. Only No. 6, 19, 20 and 27 show some asymmetry to the left. Two tumours (No. 8 and 9) however show a slight asymmetry to the right, with more hypermodal than hypomodal counts. For comparison with the DNA content, we have taken the mean chromosome number of these two tumours, the mean being in both instances one higher than the mode. No. 14, 21, 25 and 28 have no clear mode, and here the mean value of the counts in the modal range has been taken as representative of the tumour.

Brief clinical and pathological details of the cases are given in Table I, together with the modal (or mean) chromosome number, calculated to the nearest chromosome (or for tumours with less than 52 chromosomes, to the nearest half chromo-

TABLE I.—*Basic DNA Values, Expected and Observed Chromosome Numbers and DNA/Chromosome Ratios of Main Group of 30 Human Malignant Tumours.  The Data are Based on Material from the Untreated Primary Tumour, Except where Indicated*

Histology abbreviations :  A = Adenocarcinoma ;  S = Squamous cell carcinoma ;  AS = Carcinoma with both glandular and squamous elements ;  G = Good differentiation ;  M = Moderate differentiation ;  P = Poor differentiation.

| Patient number | Sex | Age | Site | Histology | Previous treatment | Basic DNA value (normal leucocyte-fibroblast value=100) | Expected chromosome number | Observed chromosome number | DNA chromosome ratio (ratio of chromosome number expected on basis of DNA content to observed chromosome number) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F. | 68 | Colon | A (M) | Surgery | 103 | 43 | 38 | 1·13 |
| 2 | F. | 49 | Cervix uteri | S (P) | Surgery | 120 | 50 | 41 | 1·22 |
| 3 | F. | 62 | Corpus uteri | A (P) | — | 98 | 41 | 41·5 | 0·99 |
| 4 | F. | 68 | Rectum | A (G) | — | 108 | 45 | 42·5 | 1·06 |
| 5 | F. | 46 | Cervix uteri | S (M) | — | 106 | 44·5 | 43·5 | 1·02 |
| 6 | F. | 57 | Corpus uteri | A (M) | — | 107 | 44·5 | 46 | 0·97 |
| 7 | F. | 81 | Colon | A (M) | — | 115 | 48 | 46 | 1·05 |
| 8 | F. | 61 | Corpus uteri | AS (M) | — | 119 | 50 | 47 | 1·06 |
| 9 | F. | 57 | Cervix uteri | S (M) | — | 123 | 51·5 | 48 | 1·07 |
| 10 | F. | 58 | Corpus uteri | A (M) | — | 119 | 50 | 48 | 1·04 |
| 11 | F. | 88 | Inguinal lymph node | Reticulum cell sarcoma | — | 113 | 52 | 49 | 1·06 |
| 12 | F. | 37 | Cervix uteri | S (P) | Stockholm Radium insertion 12 days previously | 122 | 51 | 50 | 1·02 |
| 13 | F. | 75 | Colon | A (M) | — | 124 | 52 | 51·5 | 1·01 |
| 14 | F. | 41 | Cervix uteri | S (P) | — | 140 | 59 | 53 | 1·11 |
| 15 | F. | 50 | Corpus uteri | A (M) | — | 134 | 56 | 53 | 1·06 |
| 16 | M. | 24 | Testis | Malignant teratoma | — | 126 | 52·5 | 53 | 0·99 |
| 17 | F. | 55 | Ovary | A (M) | — | 131 | 55 | 54 | 1·02 |
| 18 | F. | 67 | Malignant ascites (carcinoma of ovary) | A (P) | Intra-abdominal radio-yttrium ; chemo-therapy | 152 | 64 | 55 | 1·16 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 19 | F. | 41 | Cervix uteri | S (P) | — | 144 | 60 | 60 | 1·00 |
| 20 | M. | 59 | Rectum | A (M) | — | 155 | 65 | 60 | 1·08 |
| 21 | F. | 63 | Secondary deposit in omentum from carcinoma of ovary | A (M) | — | 163 | 68 | 60 | 1·13 |
| 22 | F. | 48 | Cervix uteri | S (P) | — | 138 | 57·5 | 60 | 0·96 |
| 23 | M. | 51 | Testis | Seminoma | — | 146 | 61 | 62 | 0·98 |
| 24 | F. | 69 | Ovary | A (G) | — | 142 | 59·5 | 62 | 0·96 |
| 25 | M. | 61 | Stomach (i) Primary tumour | A (P & M areas) | — | 157 | 66 | 64 | 1·03 |
| | | | (ii) Secondary deposit in regional lymph node | | — | 173 | 72 | 79* | 0·91* |
| 26 | M. | 72 | Colon | A (M) | — | 163 | 68 | 66 | 1·03 |
| 27 | M. | 55 | Secondary deposit in colon from carcinoma of bronchus | Compatible with 1° bronchus (P) | — | 165 | 69 | 68 | 1·02 |
| 28 | F. | 64 | Cervix uteri | S (P) | — | 169 | 71 | 71 | 1·00 |
| 29 | F. | 77 | Rectum | A (G) | — | 103 | 43 | 75 | 0·57 |
| 30 | M. | 42 | Testis | Seminoma | — | 248 | 104 | 108 | 0·96 |

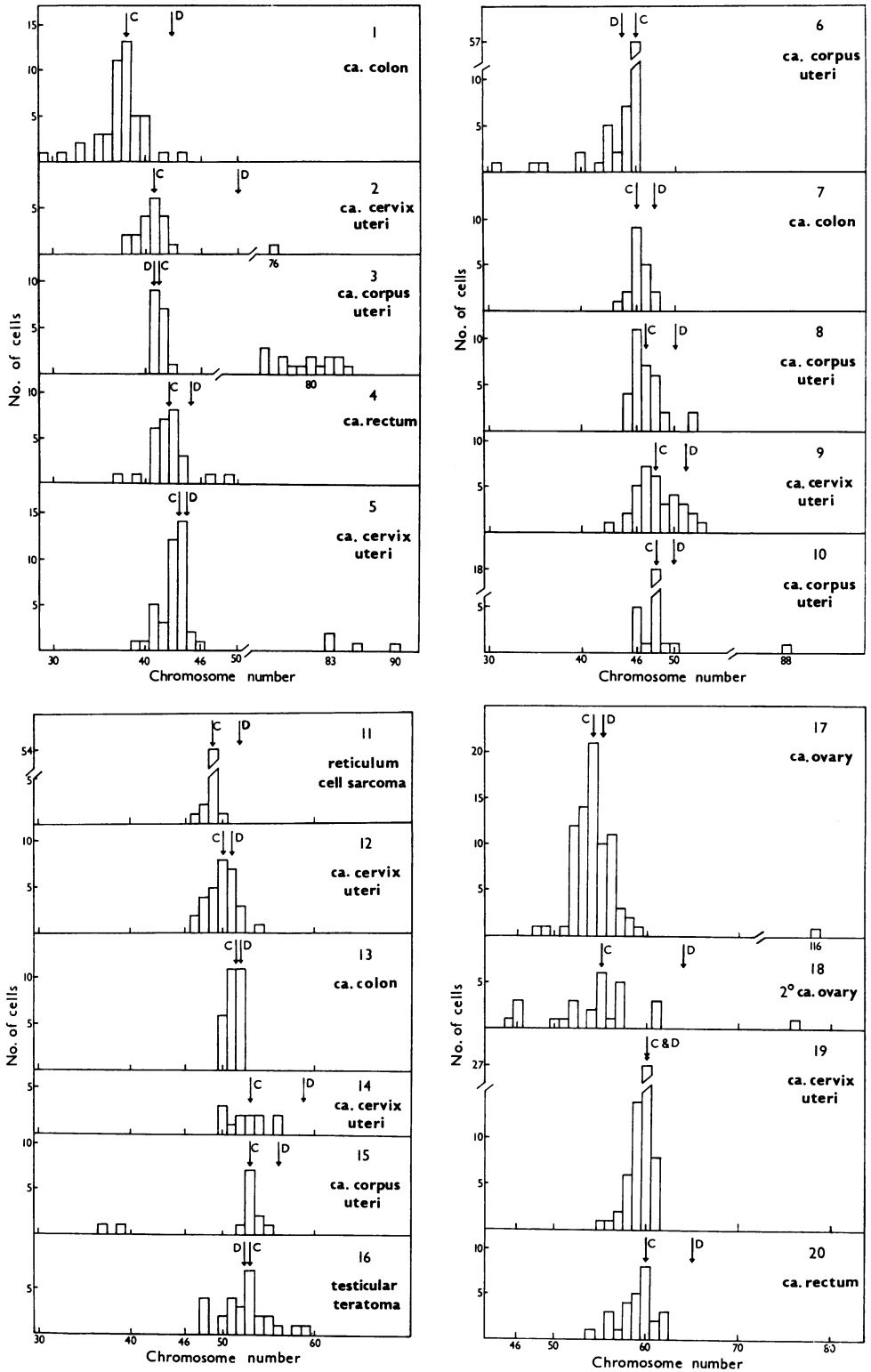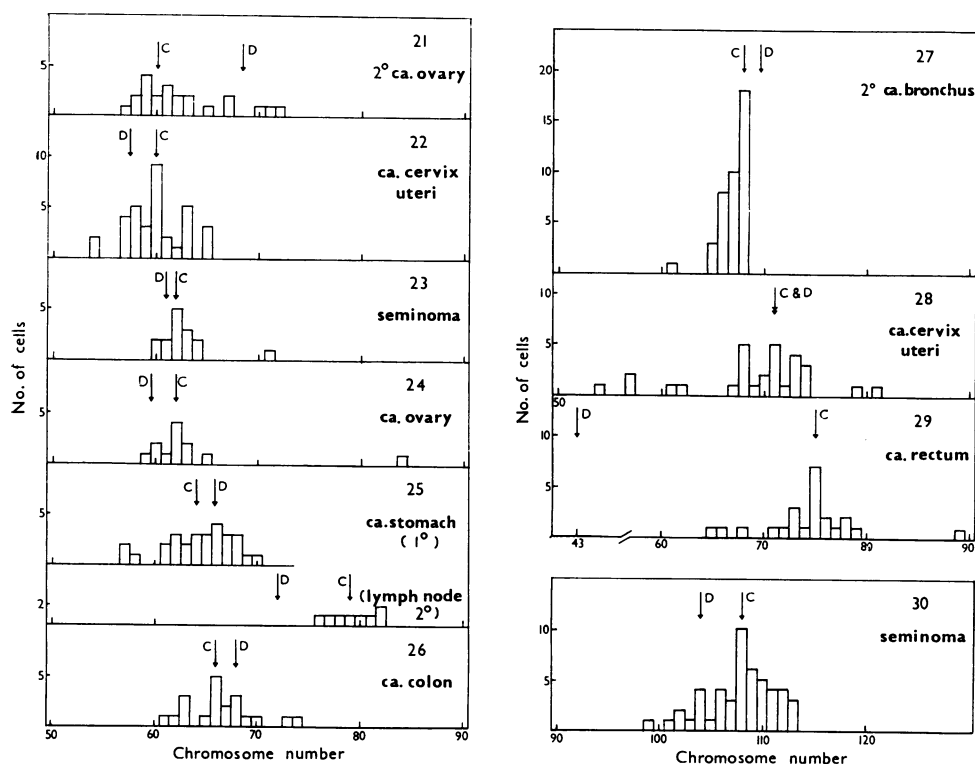* Based on 8 chromosome counts only.

FIG. 1.

FIG. 1.—Exact chromosome counts from main group of 30 tumours.  C = observed chromosome number (mode, or mean of modal range).  D = expected chromosome number, estimated from DNA measurements.

some), the basic DNA value, the expected chromosome number, and the ratio between this value and the observed chromosome number (the DNA/chromosome ratio), calculated as described in the previous section.  The modal DNA values, in terms of expected chromosome number, assuming the same mean DNA content per chromosome as for normal cells, are also indicated by arrows in the histograms (Fig. 1).  It will be seen from Fig. 1 that the expected chromosome number of most of the tumours is higher than the observed chromosome number, indicating that the mean chromosomal DNA content is greater than normal.

## B. *Second Group of* 20 *Tumours*

Distributions of exact and approximate counts, together with the other relevant data are shown in Table II.  Although there were fewer exact counts compared with the first group, it was still clear that they tended to be grouped together ; as however a mode was not clearly definable, the mean chromosome number, to the nearest whole number, was calculated.  In calculating this mean, metaphases on which approximate counts were made were regarded as having the central number of their class.

TABLE IIA.—*Exact and Approximate Chromosome Counts of Second Group of 20 Human Malignant Tumours. All are Untreated and, Excepting No. 43, the Material is from the Primary Tumour*

**Exact chromosome counts**

Patients 31–35

| Patient number | 41 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|
| 31 | 1 | — | 1 | 1 | 1 | — | 1 | 1 | — | — |
| 32 | — | 1 | 1 | 1 | 1 | 1 | — | — | 1 | — |
| 33 | — | — | — | — | — | 1 | — | 1 | 1 | — |
| 34 | — | 1 | 1 | — | — | 1 | — | — | 1 | 1 |
| 35 | — | 1 | 1 | — | — | 1 | — | — | 1 | 1 |

Patients 36–40

| Patient number | 50 | 52 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 67 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 2 | 1 | — | 1 | — | 1 | 1 | 1 | 2 | 1 | 1 | — | — | — |
| 37 | — | 1 | 1 | — | 4 | 1 | 1 | — | 2 | 2 | — | 2 | — | 1 |
| 38 | — | — | — | — | — | 1 | — | — | 2 | 2 | 2 | 1 | 2 | 1 |
| 39 | — | 1 | 1 | — | — | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 40 | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 |

Patients 41–44

| Patient number | 59 | 62 | 65 | 66 | 67 | 68 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | — | — | 2 | — | — | 1 |
| 42 | 2 | 1 | — | 1 | — | — | 1 | — | 3 | 2 | 1 | — | — | — |
| 43 | — | — | — | — | — | — | — | 3 | 1 | 4 | — | — | — | — |
| 44 | — | — | — | — | — | — | 1 | 1 | 1 | — | 2 | 2 | 1 | 1 |

Patients 45–47

| Patient number | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 80 | 81 | 82 |
|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 1 | 1 | 2 | 1 | 2 | 3 | 2 | 1 | 1 | — |
| 46 | 1 | 1 | 1 | 1 | 2 | 1 | — | 2 | 1 | — |
| 47 | — | — | — | — | 2 | 1 | — | 3 | 3 | 5 |

Patients 48–49

| Patient number | 83 | 84 | 86 | 87 | 88 | 90 | 91 |
|---|---|---|---|---|---|---|---|
| 48 | 1 | 1 | — | 1 | 1 | 1 | 1 |
| 49 | 1 | 3 | — | 2 | 2 | — | — |

Patient 50

| Patient number | 111 | 112 | 124 | 127 | 129 | 135 |
|---|---|---|---|---|---|---|
| 50 | 1 | 1 | 1 | 1 | 1 | 2 |

**Approximate chromosome counts**

Patients 31–40

| Patient number | 41–45 | 46–50 | 51–55 | 56–60 | 61–65 | 66–70 |
|---|---|---|---|---|---|---|
| 31 | 2 | 10 | — | — | — | — |
| 32 | 1 | 7 | 1 | — | — | — |
| 33 | — | 4 | 1 | 1 | — | — |
| 34 | 1 | 3 | 1 | — | — | — |
| 35 | 5 | 9 | 3 | 2 | 1 | 1 |
| 36 | 1 | 2 | 1 | 2 | — | — |
| 37 | — | — | 1 | 5 | 2 | — |
| 38 | — | — | 1 | — | 2 | — |
| 39 | — | — | — | 2 | 1 | — |
| 40 | — | — | — | — | — | — |

Patients 41–44

| Patient number | 56–60 | 61–65 | 66–70 | 71–75 | 76–80 | 81–85 | 86–90 |
|---|---|---|---|---|---|---|---|
| 41 | 1 | 6 | 7 | 3 | — | 1 | 1 |
| 42 | 2 | 1 | 2 | 3 | 1 | — | — |
| 43 | — | — | — | — | — | — | — |
| 44 | — | — | — | 1 | — | — | 1 |

Patients 45–47

| Patient number | 71–75 | 76–80 | 81–85 | 86–90 | 91–95 |
|---|---|---|---|---|---|
| 45 | 1 | — | — | 2 | 1 |
| 46 | 8 | 7 | — | — | — |
| 47 | — | 3 | 6 | — | — |

Patients 48–49

| Patient number | 81–85 | 86–90 | 91–95 |
|---|---|---|---|
| 48 | — | — | 1 |
| 49 | 11 | 2 | — |

TABLE IIB.—*Basic DNA Values, Expected and Observed Chromosome Numbers and DNA/Chromosome Ratios of Second Group of 20 Human Malignant Tumours. All are Untreated and, Excepting No. 43, the Material is from the Primary Tumour*

Histology abbreviations : A = Adenocarcinoma ; S = Squamous cell carcinoma ; AS = Carcinoma with both glandular and squamous elements ; G = Good differentiation ; M = Moderate differentiation ; P = Poor differentiation.

| Patient number | Sex | Age | Site | Histology | Basic DNA value | Expected chromosome number | Observed chromosome number | DNA chromosome ratio (ratio of chromosome number expected on basis of DNA content to observed chromosome number) |
|---|---|---|---|---|---|---|---|---|
| 31 | F. | 68 | Cervix uteri | S (M) | 116 | 48·5 | 46 | 1·05 |
| 32 | F. | 51 | Cervix uteri | S (M) | 130 | 54 | 48 | 1·13 |
| 33 | F. | 76 | Cervix uteri | S (P) | 122 | 51 | 50 | 1·02 |
| 34 | F. | 54 | Cervix uteri | S (G) | 121 | 50·5 | 50 | 1·01 |
| 35 | F. | 43 | Cervix uteri | S (P) | 121 | 50·5 | 51 | 0·99 |
| 36 | F. | 54 | Cervix uteri | AS (P) | 146 | 61 | 53 | 1·15 |
| 37 | F. | 53 | Cervix uteri | S (P) | 160 | 67 | 59 | 1·14 |
| 38 | F. | 75 | Cervix uteri | A (P) | 176 | 74 | 59 | 1·25 |
| 39 | F. | 52 | Cervix uteri | S (M) | 144 | 60 | 59 | 1·02 |
| 40 | F. | 56 | Colon | A (G) | 148 | 62 | 64 | 0·97 |
| 41 | F. | 68 | Colon | A (M) | 164 | 68·5 | 67 | 1·02 |
| 42 | M. | 49 | Cervix uteri | S (G) | 200 | 83·5 | 69 | 1·21 |
| 43 | M. | 51 | Secondary deposit in cervical lymph node from carcinoma of bronchus | ? (P) | 176 | 74 | 73 | 1·01 |
| 44 | F. | 68 | Breast | A (P) | 196 | 82 | 75 | 1·09 |
| 45 | F. | 54 | Cervix uteri | S (P) | 187 | 78 | 76 | 1·03 |
| 46 | M. | 63 | Rectum | A (M) | 170 | 71 | 77 | 0·92 |
| 47 | F. | 73 | Cervix uteri | A (M) | 206 | 86 | 81 | 1·06 |
| 48 | M. | 73 | Colon | A (M) | 176 | 74 | 86 | 0·86 |
| 49 | M. | 44 | Colon | A (M) | 202 | 84·5 | 88 | 0·96 |
| 50 | F. | 50 | Colon | A (G) | 328 | 137 | 125 | 1·10 |

*The relation between DNA content and chromosome number*

Considering first the main group of 30 tumours on which substantial numbers of chromosome counts were made, it is evident that except for Tumour No. 29 there is quite good agreement between basic DNA content and chromosome number. For Tumour No. 29, however, the observed chromosome number is approximately twice that expected from the DNA data. This discrepancy is possibly due to the fact that in the region selected for chromosome counts most of the cells had doubled their chromosome complement, whereas in the region on which DNA measurements were made there was only a moderate degree of polyploidization of the " basic " cell-line (a varying degree of polyploidy in different regions of some human tumours has previously been inferred from data on DNA content and nuclear size (Atkin, 1962)). DNA estimations were therefore made on the material, which had been pretreated for chromosome spreading, from this region : although the preparations were less satisfactory from the point of view of identification of cell-types because of the hypotonic pretreatment and air-drying, it was clear that the majority of interphase tumour cells in this region of the tumour had near-tetraploid DNA contents.

Excluding Tumour No. 29, and the metastasis of Tumour No. 25 which yielded only a few chromosome counts, the mean DNA/chromosome ratio of the remaining 29 tumours is 1·040 (standard deviation ±0·063). If we consider only the 29 untreated primary carcinomas and teratoma (excluding the two seminomas because of uncertainty regarding the DNA content of the homologous normal cells, i.e. whether, like epithelial cells, they have a 10% higher content than leucocytes), we find a mean DNA/chromosome ratio of 1·026 (standard deviation ±0·042). On the other hand 4 out of the 5 tumours (No. 1, 2, 18, 21 and 27) which were either metastases or recurrences following treatment had DNA/chromosome ratios greater than 1·1. The DNA/chromosome ratios are shown graphically in Fig. 2.

The data on the 20 tumours in the second group (Table II and Fig. 2) also show a tendency for the tumours to have more DNA per chromosome than normal. There is however slightly greater variation in the DNA/chromosome ratios than in the first group, probably because fewer chromosome counts were made. The mean DNA/chromosome ratio of the group is 1·049 (standard deviation ±0·10).

*A comparison of the DNA/chromosome ratios and the karyotypes of* 17 *tumours*

Karyotype analyses have been performed on 17 of the tumours in the present study, and it is of interest to compare the karyotypes with the DNA/chromosome ratios. Karyotypes of 4 of the tumours have been illustrated in a separate publication (Atkin and Baker, 1966) : No. 11 (Fig. 4) ; No. 17 (Fig. 2) ; No. 18 (Fig. 1) ; and No. 19 (Fig. 3). Brief details of the karyotypes of the following tumours are also given in Table 6 of the same publication, the numbers in brackets being those given to the tumours in this table: No. 1 (6), 2 (7), 4 (9), 5 (8), 6 (10), 8 (11), 9 (12), 10 (13), 13 (14), 15 (16), 16 (15) and 23 (17). Tumour No. 6 was exceptional in that most of the tumour-cell metaphases had apparently normal karyotypes. Although the other tumours showed varying degrees of aneuploidy, all the chromosome groups were usually represented, and no very great departure from normal with respect to the mean DNA content per chromosome was therefore to be expected. However, as a possible indication of a change in the DNA per

chromosome, we have taken a representative karyotype from each of the 17 tumours on which analyses have been performed (all except No. 23 were from female patients) and counted the numbers of " large ", " medium " and " small " chromosomes in each karyotype (the " medium " group comprises the C group
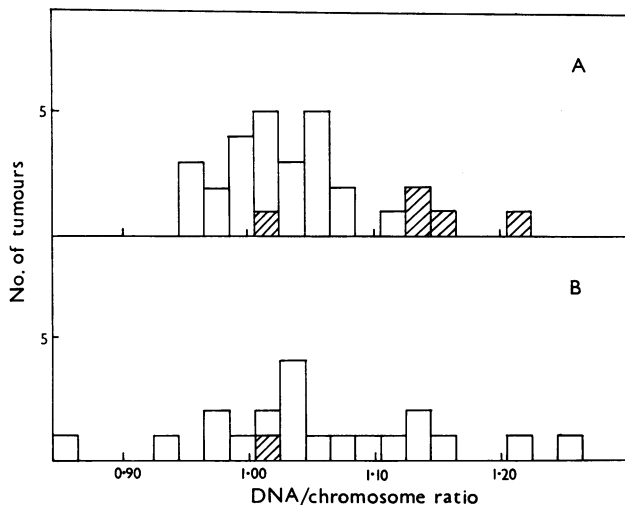


FIG. 2.—DNA/chromosome ratios of (A) 29 tumours from the main group (excluding No. 29 and the lymph node metastasis of No. 25) and (B) second group of 20 tumours. The shaded areas represent recurrent or metastatic tumours.
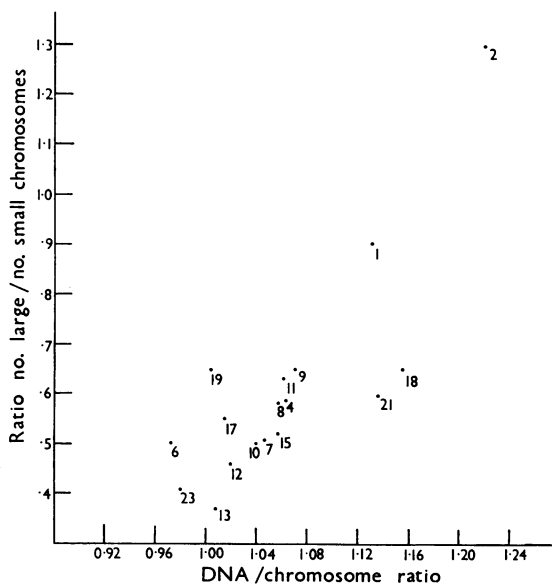


FIG. 3.—DNA/chromosome ratios and ratios of number of large to number of small chromosomes (derived from representative karyotypes as explained in the text) of 17 tumours from the main group.

chromosomes and any abnormal chromosomes within the size-range of the C group chromosomes). The ratio of the number of large to number of small chromosomes (ignoring the medium-size chromosomes, since these are close to the mean size) should provide some indication of changes in the average size of chromosomes in the karyotype. This ratio for normal female cells is:

$$\frac{\text{A and B group chromosomes}}{\text{D, E, F and G group chromosomes}} = \frac{10}{20} = 0.5.$$

As can be seen from Fig. 3, the majority of the ratios are greater than 0·5, indicating relatively more large than small chromosomes compared with normal cells, and furthermore there appears to be a correlation between this ratio and the DNA/chromosome ratio.

<div align="center">DISCUSSION</div>

Early studies on the DNA contents of human tumour cells using microspectrophotometric techniques (Leuchtenberger, Leuchtenberger and Davis, 1954; Atkin and Richards, 1956) revealed that the interphase cells were usually grouped around a mode which was either near the normal diploid level, or else near the tetraploid level. These modes corresponded to near-diploid and near-tetraploid chromosome numbers respectively, provided that the cells contributing to the mode had not yet commenced to replicate their DNA, and that the mean DNA content per chromosome was about the same as that of normal cells. However, at the time these studies were made, techniques for studying the chromosomes of solid tumours had not yet been developed, and it was impossible to verify whether in fact the DNA mode of a tumour showed any correspondence to the chromosome number. In a later study based on a series of 14 human malignant tumours which were especially favourable for chromosome analysis, the DNA content and chromosome number were in more or less close agreement (Richards and Atkin, 1960); on the average, however, the DNA content exceeded that expected on the basis of the chromosome number by about 14%, and it thus appeared that there might be a tendency for tumours to have a rather larger mean chromosome size than normal cells. Up to the time of the present study, no more detailed investigation on the relation between DNA content and chromosome number has been reported. Ojima, Inui and Makino (1960) found agreement between the DNA content and approximate chromosome number of 4 uterine tumours, but a precise comparison was not attempted.

The present study has revealed a more or less close correlation between DNA content and chromosome number for all except one of the fifty human tumours, although there is frequently a small discrepancy which is usually in the direction of more DNA per chromosome than normal cells. The variation in the DNA/chromosome ratio that we have found for different tumours is no doubt partly due to sampling errors. The finding that the ratio is on the average about 1·04 rather than 1 might possibly be due to an undetermined systematic error. However, the observations on the karyotypes of 17 of the tumours provide confirmatory evidence that the majority of these tumours do in fact have on the average larger chromosomes than normal cells.

It may be instructive to consider the conditions which appear necessary for the demonstration of the correlation that we have found in the present study. For such a correlation to be present, it would appear that:

(1) Most of the interphase cells must have similar DNA contents, i.e. the values are grouped around a clearly-definable mode (the microspectrophotometric technique should of course be of sufficient accuracy, otherwise a mode even if present might not be apparent from the frequency distribution of individual measurements).

(2) This mode is formed by cells which have not commenced DNA synthesis and still have the " single " amount of DNA ; it is not significantly distorted by the accidental inclusion of cells which have slightly increased DNA contents because they have commenced DNA synthesis.

(3) There is likewise a definable chromosome number, around which most of the chromosome counts on cells in metaphase are grouped.

(4) There is a degree of consistency within each tumour : since DNA measurements and chromosome counts on the solid tumours have been made on separate pieces of tumour, the good agreement found for nearly all the tumours suggests that generally the interphase cells in the one specimen and the metaphases in the other have identical or almost identical chromosome complements. The lack of correlation found for Tumour No. 29 indicates that there is variation within this tumour, but, as suggested above, this variation may be merely with regard to the degree of polyploidy ; it is also of interest that although the DNA data and chromosome counts obtained from the primary region of Tumour No. 25 were in mutual agreement, as were those from the metastasis, the two regions (primary and metastasis) were significantly different from each other. Examples from the literature of human tumours and malignant effusions having more than one modal chromosome number or DNA value have been cited elsewhere (Atkin and Baker, 1966). However, our results suggest that mosaicism, with cell-lines which differ widely with respect to DNA content and chromosome number, is uncommon, at least in primary untreated tumours.

Our results indicate that the above-enumerated conditions generally obtain, and the generally close agreement between the DNA data on interphase cells and the chromosome counts (the exception being explainable on the basis of regional variation within the tumour) leaves little doubt that DNA estimations on interphase cells can provide a quite accurate estimate of the total chromosomal content of a tumour cell-line karyotype. Observations on the karyotypes, where available, have strenghtened the conclusion that the DNA content has indeed changed more or less in proportion to the chromosome number ; in fact, the ratios of number of large to number of small chromosomes, summarized for 17 of the tumours in Fig. 3, have provided a useful cross-check for the observation that the average DNA per chromosome is close to but frequently slightly greater than that of normal cells.

Together, the DNA data and the observations on the chromosome numbers and karyotypes of malignant cells reported here suggest the following generalizations concerning the chromosomes of human tumours. Although apparently at random, the karyotype changes in malignant cell-lines occur within certain more or less well-defined limits. It is of interest that tumours sometimes have less than the normal diploid content of genetic material. The tumour (No. 1) with the lowest chromosome number (38) in the present series has a DNA content that is about 6% less than the normal diploid value. However, the amount of genetic material is usually increased. Tumour No. 50 has the highest chromosome number (125), and its DNA content is proportionately even higher. Although

tumours may thus vary within wide limits, it is nevertheless apparent from DNA data on a large series of tumours that certain DNA levels are favoured. Thus, among an unselected series of 202 squamous-cell carcinomas of the cervix, it was found from DNA data that near-diploid and near-tetraploid (usually hypotetraploid) tumours were about equally common, whereas near-triploid tumours were slightly less so ; on the other hand, tumours with near-diploid DNA modes predominated in a series of 51 carcinomas of the corpus uteri, although there was also a small near-tetraploid group (Atkin, 1964a). The DNA content of uterine tumours may be of prognostic significance : although most patients have been followed up for less than 5 years, the data suggest a relatively more favourable prognosis for tumours of the cervix uteri if the DNA mode is in the hypotetraploid region and for tumours of the corpus uteri if it is in the diploid region (Atkin, 1964a ; Atkin, as yet unpublished data).

The observation that tumours frequently have on the average slightly larger chromosomes than normal cells may perhaps reflect certain laws with regard to the changes that have occurred in the malignant karyotype. Thus, larger chromosomes may be preferentially gained or smaller chromosomes lost. Structural changes may result in the formation of two unequal-sized chromosomes, of which the smaller is lost. It is of interest that an abnormal chromosome is sometimes encountered in human tumours that is larger than any of the normal set—for instance in Tumour No. 18 : see Atkin and Baker (1966), Fig. 1.

The high DNA/chromosome ratio of 4 out of the 5 treated or metastatic tumours poses the question of whether a high ratio is a common feature of recurrences or metastases and if so whether a similarly high ratio is present in the corresponding primary tumour, or whether a change has occurred with the onset of the recurrence or metastasis.

Although there may thus be a small discrepancy, the observation that the DNA content of human tumours generally bears a more or less close relation to the chromosome number is in keeping with the view that some degree of balance must be maintained in the karyotype. Observations on tumour-cell karyotypes show that most of the normal chromosome groups are represented, and abnormal chromosomes, although present in many tumours, rarely appear to exceed 3 or 4 in number ; these abnormal chromosomes may as noted above be larger than (or sometimes smaller than) any of the normal chromosomes, but usually they are within the normal size-range (Atkin and Baker, 1966). It is of interest to compare the situation in tumours with that found in a representative series of 6 placental mammals (Atkin, Mattinson, Beçak and Ohno, 1965) : despite a more than fourfold variation in the diploid chromosome number, the mean DNA content per cell varied by not more than about 10%. Obviously, extensive structural changes, without significant alterations in the total chromosomal content, have occurred during the evolution of these species and have led to the wide differences in chromosome number. In the transition to malignancy, however, there have been relatively few structural changes, and although the number of chromosomes varies widely the range and distribution of chromosome sizes in human tumour cells correspond fairly closely to those of normal cells.

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SUMMARY

1. The modal DNA content (estimated by Feulgen microspectrophotometry of interphase cells) and the chromosome number of 49 human malignant tumours were in fairly close mutual agreement. In a further tumour, the chromosome number was about double that expected on the basis of DNA measurements ; this discrepancy may have resulted from variation with regard to the degree of polyploidy within the tumour, the majority of cells in the region selected for chromosome counts having undergone polyploidization.

2. In the 49 tumours showing reasonably good agreement, there was frequently a small discrepancy between DNA content and chromosome number : the expected chromosome number (based on the DNA data, assuming the same mean DNA content per chromosome as for normal cells) exceeded the observed chromosome number on the average by about 4%.

3. Karyotype observations on 17 of the tumours supported the evidence from DNA measurements that the average chromosome size in the tumour cell karyotypes was usually greater than normal.

4. The findings are briefly discussed in relation to the wide and apparently random variation in the karyotypes of different tumours.

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