Cytological basis of histological typing of diffuse Hodgkin's disease

Demonstration of an implied misnomer in the terminology of the Rye classification

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SUMMARY Differential cell counts were made on nine lymph nodes whose structure was replaced by diffuse Hodgkin's disease; two of these nodes had the classical histological appearance of the lymphocytic predominance subtype, four of the mixed cellularity subtype, and three of the lymphocytic depletion subtype. Our attempts to achieve valid sampling methods are recorded. The counts, in general, confirm the postulated histological basis of the Rye classification of the subtypes of the diffuse disease. The major discrepancy is that, contrary to the histological descriptions, our direct counts have shown that lymphocytes are, in general, more numerous in the lymphocytic depletion than in the mixed cellularity subtypes. The cell counts also show that normal mononuclear cells (mainly fibroblasts and macrophage-type cells) are much more numerous in the mixed cellularity subtype than in the other forms of diffuse Hodgkin's disease; this feature has not been emphasised in the Rye classification.

On the basis of our differential counts, a hypothesis is proposed that could explain the natural history of the different subtypes of diffuse Hodgkin's disease as the resultant of three processes: (a) tumour aggressiveness, (b) specific cell-mediated immunological reactions, and (c) non-immunological stromal responses.

The lesions of Hodgkin's disease are composed of many different cell types; these include lymphocytes, plasma cells, neutrophil and eosinophil granulocytes, histiocytes, fibroblasts, atypical mononuclear cells, and atypical multinuclears, including Reed-Sternberg cells.

The disease is unpredictable in the rate at which it progresses and in the manner in which it responds to treatment. Furthermore, there is great variation in the histological structure of the lesions in different patients. Consequently, there has been continuing interest in histological classifications that purport to give an indication of prognosis. Currently, the most widely used scheme is the Rye Conference (Lukes *et al.*, 1966) simplification of the Lukes and Butler (1966) classification. The four subtypes that are identified are (a) the *lymphocytic predominance* subtype, which, as its name suggests, usually contains many lymphocytes and relatively few

abnormal mono- and multinuclear cells; however, some cases classified in this way show predominance of reactive histiocytes; (b) the *lymphocytic depletion* subtype, which is characterised either by cellular depletion and fibrosis or by the presence of large numbers of Reed-Sternberg cells and other abnormal mono- and multinuclear cells with or without lymphocyte depletion; (c) the *mixed cellularity* subtype, which is defined as being intermediate in histological appearance between the lymphocytic predominance and lymphocytic depletion subtypes; (d) the *nodular sclerosis* subtype, which is characterised by orderly bands of interconnecting collagenous connective tissue that subdivide the abnormal lymphoid tissue into isolated cellular nodules.

Inherent in the definition of these subtypes are two assumptions: (1) that the lesions may be diffuse (homogeneous) or nodular (focally heterogeneous), and (2) that the differences in the histological appearances are a consequence of substantial

Received for publication 1 December 1977

variation in the overall cell density and in the relative proportions of the different cell types. Neither of these assumptions has been established in quantitative terms, although the crudely semi-quantitative studies of Coppleson *et al.* (1973) do not contradict the second assumption. This paper describes for the first time the extent of variation in the cellular composition of diffuse forms of Hodgkin's disease in truly quantitative terms.

Material and methods

Paraffin-embedded blocks of lymph-node tissue from nine known cases of Hodgkin's disease were studied; one lymph node was obtained at necropsy, the others were surgical specimens. These blocks were selected by a pathologist (JBMacG) specially interested in lymphoid neoplasms as typical examples of the three diffuse subtypes in the Rye classification.

Fixation, processing, and staining methods followed standard procedures; the nodes obtained surgically or at necropsy were bisected and fixed in 4% neutral buffered formaldehyde followed by secondary fixation in formol sublimate, and advanced to paraffin via an automatic tissue processor.

From each lymph node eight serial sections were cut at a micrometer setting of 7 μm . A Surfometer (Planer Products Ltd, Sunbury-on-Thames, Middlesex; Type SF100) was used to measure accurately the section thickness (Pearse and Marks, 1974). This instrument consists of a displacement transducer, which monitors the vertical movement of a diamond stylus as it traverses the surface of the section mounted on a microscope slide. The measured section thickness varied from 7 to 8 μm . The first, fourth, and seventh sections from each block were stained with Mayer's haemalum, counterstained with eosin, and mounted in synthetic resin. Intermediate sections from all nodes were stained with Mayer's haemalum alone.

The sections were examined under a microscope with a 40 $\times$ objective and 12.5 $\times$ eyepiece (total magnification 500 $\times$); the focusing eyepiece contained a 10 $\times$ 10 mm graticule divided into 25 equal squares (Leitz, 519902). With this optical system, the area included within each small graticule square was 0.003969 mm². A major problem in differential counting of cells in the Hodgkin's disease lesions is the great variety of constituent cell types, some of which can be difficult to distinguish with confidence. Consequently, cells were classified into six readily identifiable categories. Three of them, (a) lymphocytes, (b) plasma cells, and (c) polymorphs (including neutrophil and eosinophil granulocytes), did not prove difficult to recognise. The other three classes were (d) normal mononuclears, a category

that included fibroblasts, reactive histiocytes, and endothelial cells as well as other non-neoplastic cells that could not be identified in categories (a) to (c) above; (e) abnormal mononuclears, some of which had a large vesicular nucleus with prominent nucleolus whereas others had more irregular hyperchromatic nuclei; and (f) abnormal multinuclears, a group which included Reed-Sternberg cells. Two observers with limited histological experience were trained by a consultant pathologist to identify the cells in lesions of Hodgkin's disease according to this classification; in general, they found the system workable, but at times they had considerable difficulty in distinguishing (a) lymphocytes from small mononuclears and (b) normal from abnormal mononuclears, consequently some arbitrary decisions had to be made. However, subsequent retesting showed that these observers consistently allocated the cells to the same category as their instructor. In the course of the main investigation the two observers used somewhat different sampling methods in an attempt to obtain an unbiased estimate of the cell content of the Hodgkin's disease lymph nodes.

Method A was applied to sections stained with haemalum and eosin. Several areas, each approximately 2.5 mm², were marked at random with Indian ink on the coverslip over the section. Each area was then traversed regularly, using the eyepiece graticule as a guide so that individual microscope fields were contiguous without overlap or separation. The number of cells of each of the six types was counted in each of the 13 alternate small squares within each graticule field, using the convention that a cell falling within or on the right or lower border of a square was included, while any cell falling on the left or upper border was ignored. Thus, by systematic traverse, the marked area was sampled. The optimum method of sampling was then determined by comparing the counts accumulated from the marked area in three ways, (1) all 13 small squares, (2) the five small squares at the corners and centre of the graticule field, and (3) the one small square at the centre of each graticule field or at top left-hand or bottom left-hand corner.

Method B was applied to sections stained with haemalum alone. A clear film bearing a photographed 0.5 mm² grid was secured to the upper surface of the coverslip over the section. To avoid histological bias in the selection of the area to be sampled four of these squares were chosen for differential counting by naked eye before the section was examined microscopically. The grid square was then traversed as in method A. Cells in all the graticule squares within the grid square were

counted, apart from those lying in the zone of optical diffraction at the edges of the photographed lines where cells could not be identified with confidence. In all, the content of 49 small graticule squares was counted in each selected area.

Results

DETERMINATION OF OPTIMUM APPROACH TO SAMPLING IN COUNTS WITH METHOD A

The counts on a section of lymph node diagnosed as lymphocytic depletion subtype of Hodgkin's disease from patient 7 are shown in Table 1. The counts on 13 small squares per graticule field were used to estimate the expected numbers of each type of cell per small square together with their standard deviations. The consistency of the counts on five or one small square per graticule field with the larger sample was checked by calculating the ratio

$$\frac{\text{observed no.} - \text{expected no.}}{\text{standard deviation}}$$

for each type. A count is inconsistent with the larger sample when the ratio exceeds 1.96 (Poisson dispersion test). In fact for all the one- or five-small square counts, only one ratio, that of the number of lymphocytes counted when the sampled area was restricted to the top left-hand corner, *exceeded* the critical value of 1.96. It was therefore concluded that counts in one small square per graticule field would yield a satisfactory sample of the population of cells in this section of lymphocytic depletion Hodgkin's disease.

Similar studies on a lymphocytic predominance node (patient 1) indicated that only one small square per graticule field needed to be counted in this section. However, there was greater variation in the mixed cellularity node (patient 3), and valid results were obtained only when five small squares were counted per graticule field.

COMPARISON BETWEEN THE TWO COUNTING METHODS

Lymph nodes from three patients (1, 3, and 7) were counted by both methods; one observer used method A and the other method B. Each observer made differential counts on four marked areas of the section from each patient; they studied different sections because of the requirement for different staining methods. The means of the numbers of cells of each type per small square were compared using Student's *t* test. No appreciable differences were detected for any cell type in the mixed cellularity lymph node or for lymphocytes or abnormal multinuclears in the lymphocytic predominance lymph node; small, but statistically significant ($P < 0.05$), differences were found for all cell types in the lymphocytic depletion lymph node and for both normal and abnormal mononuclears in the lymphocytic predominance lymph nodes.

The differences between the cell counts obtained with the two methods are significant in the statistical sense, but in the interpretation of the data it is important to remember that for any one case there is very little difference in the relative numbers of cells of different types counted by the two methods. Certainly the extent of the differences, although apparently real, are not great in histological terms. The maximum difference observed was 10 lymphocytes per small graticule square in the lymphocytic depletion node, and the minimum difference was about half an abnormal mononuclear per small graticule square in the lymphocytic predominance node.

CELL COUNTS IN LYMPH NODES AFFECTED BY DIFFUSE SUBTYPES OF HODGKIN'S DISEASE

The findings are summarised in Table 2, where the results are expressed as mean numbers of cells of each type per small graticule square.

Table 1 Comparison of differential cell counts within 13, 5, and 1 small graticule square per graticule field in a section of a lymph node from a patient with lymphocytic depletion Hodgkin's disease to determine optimal size of an unbiased sample

Cell type	Number of cells of each type counted when method of sampling each graticule field was:				
	13 alternate small squares	5 small squares (centre and corners)	1 small square		
			Top left-hand corner	Centre	Bottom left-hand corner
Lymphocyte	2569	1006	169	209	206
Plasma cell	2	0	0	0	0
Polymorphonuclear granulocyte	221	77	12	15	12
Normal mononuclear	694	247	56	47	45
Abnormal mononuclear	663	268	59	56	52
Abnormal multinuclear	74	29	4	7	8

Table 2 Numbers of cells of various types in lymph nodes showing different histological patterns of diffuse Hodgkin's disease

Patient	Histological classification (Rye subtype)	Method of counting	Total No. of cells counted	Mean No. (and percentage) of cells of given type per small square within a graticule field						
				Total, all types	Lymphocyte	Plasma cell	Polymorphonuclear granulocyte	Normal mononuclear	Abnormal mononuclear	Abnormal multinuclear
1	Lymphocytic predominance	A	22 026	86.49 (100)	83.98 (97)	0.03 (0)	0.21 (0.2)	1.93 (2.2)	0.23 (0.3)	0.11 (0.1)
		B	18 267	93.18 (100)	87.50 (93.9)	0.02 (0.02)	0 (0)	4.81 (5.17)	0.76 (0.81)	0.09 (0.10)
		B	19 114	97.52 (100)	93.25 (95.67)	0.57 (0.58)	0 (0)	3.09 (3.17)	0.47 (0.49)	0.04 (0.04)
3	Mixed cellularity	A	27 497	44.63 (100)	15.88 (35.59)	0 (0)	1.68 (3.77)	25.48 (57.09)	1.57 (3.51)	0.02 (0.04)
		B	7 368	37.59 (100)	15.69 (41.63)	0.05 (0.13)	0.05 (0.13)	20.22 (53.79)	1.48 (3.96)	0.12 (0.32)
		B	8 334	42.52 (100)	17.01 (40.0)	0.025 (0.05)	0.1 (0.25)	23.87 (56.15)	1.27 (2.99)	0.22 (0.52)
5	"	B	8 263	42.15 (100)	13.01 (30.86)	0.28 (0.66)	0.21 (0.52)	27.53 (65.30)	1.19 (2.83)	0.22 (0.53)
		B	9 790	49.94 (100)	8.3 (16.62)	0.06 (0.13)	0.06 (0.13)	31.17 (62.43)	7.07 (14.16)	0.28 (0.56)
		B	7 790	38.06 (100)	25.56 (67.20)	0.01 (0)	1.65 (4.3)	5.64 (14.8)	4.57 (12.0)	0.63 (1.6)
7	Lymphocytic depletion	A	15 126	33.05 (100)	14.93 (48.22)	0 (0)	0.01 (0.04)	9.36 (28.84)	7.51 (22.7)	1.22 (3.7)
		B	6 479	29.63 (100)	18.21 (61.45)	0.05 (0.17)	0.29 (0.98)	5.47 (18.48)	5.06 (17.07)	0.52 (1.77)
		B	5 809	34.72 (100)	24.33 (70.07)	0 (0)	0.05 (0.14)	4.71 (13.56)	5.18 (14.92)	0.44 (1.27)

(a) Overall cellularity

The lymphocytic predominance subtype (86-97 cells per small graticule square) contained many more cells than either the mixed cellularity (38-47 cells per small square) or lymphocytic depletion (30-38 cells per small square) subtypes. The cells were more closely packed together in the lymphocytic predominance lymph nodes than in the other two subtypes, but the difference in overall cellularity is largely explained by the greater number of larger cells in the mixed cellularity and lymphocytic depletion lymph nodes.

(b) Differential cell counts

Within each of the diffuse subtypes of Hodgkin's disease the distribution of the differential cell counts is fairly similar, and each of the subtypes shows some distinctive feature. In the lymphocytic predominance nodes the lymphocyte (84-93 per small graticule square) is the most common class of cell (94-97%), while in the mixed cellularity nodes the normal mononuclear (20-31 per small graticule square) is the most common group of cells (53-65%). Abnormal mono- and multinuclear cells are more common in lymphocytic depletion nodes (14-26%) than in lymphocytic predominance (0.4-0.9%) or mixed cellularity nodes (3.4-14.7%). Figures 1 and 2 show that, while there is marked clustering of results within each of the subtypes, there is also a suggestion of continuity between the different

diffuse subtypes of Hodgkin's disease. Figure 1 also shows, rather surprisingly, that lymphocytes are more common in lymphocytic depletion than in mixed cellularity subtypes.

REGIONAL VARIATION IN CELL COUNTS IN DIFFUSE SUBTYPES OF HODGKIN'S DISEASE

The lymph nodes from patients 1, 3, and 7 were selected for this study on the basis of apparent uniformity of histological appearances within the lesion. The variation in overall cellularity and in differential counts within different areas of the section and between semi-serial sections is shown in Table 3, the means and standard deviations being given where appropriate. The lesions of the lymphocytic predominance subtype show considerable uniformity; by contrast the lesions of the mixed cellularity and lymphocytic depletion subtypes show proportionately greater local variability.

Discussion

In this study we have attempted to characterise the histological appearances in the lesions of Hodgkin's disease by counting the numbers of cells of different types per unit area. Since the various types of cell differ considerably in size, it would not be valid to deduce the volume proportions of the various components of the Hodgkin's disease

Fig. 1 Comparison of relative number of lymphocytes with that of abnormal mono- and multinuclear cells in areas A-Z (see Table 3) counted with method A, and in areas counted with method B (see Table 2). The lymphocytic predominance (▲) nodes form a distinctive cluster. The mixed cellularity (■) and lymphocytic depletion (●) nodes show considerable overlap; rather surprisingly, lymphocytes are often more numerous in the lymphocytic depletion subtypes.

Fig. 2 Comparison of relative number of normal mononuclear cells with that of abnormal mono- and multinuclear cells in the areas identified in the legend to Fig. 1. The histological subtypes are more clearly separated than in the previous figure. Normal mononuclear cells are most numerous in the mixed cellularity subtype (■); abnormal cells are most numerous in the lymphocytic depletion subtype (●); both of these cell types are relatively infrequent in the lymphocytic predominance subtype (▲).

lesion from our data since this was not a histometric study. Because of the enormous numbers of cells even in a single section of a lymph node it has not been practicable for us to make comprehensive counts and consequently we have devised two sampling techniques. We have presented evidence on the precision and comparability of the two techniques. It is hardly surprising that relatively minor inconsistencies were found, since the two methods differed in approach to sampling and staining methods; furthermore, separate sections were used in the two methods and the counts were made by different observers. It is probable that a major cause of the disagreement was the regional variation in the structure of Hodgkin's disease lesions, since the differences were proportionately least marked (and not statistically significant) in the counts on the mixed cellularity lymph node where the five-square sampling method was used in method A to compensate for the recognised increased heterogeneity in this lesion.

The only previous reports (Coppleson *et al.*, 1970, 1973), of which we are aware, of attempts to quantitate the relative frequency of the various cell types in Hodgkin's disease were based on the visual impressions (without any actual counts) of three observers who recorded their results on a six-point scale covering the range 0-100%. The range within each point on this scale was broad; for example, their scale point 4 was presumed to be 51-80% for lymphocytes and 31-50% for all other cell types. Even so, the observers disagreed by one point in their estimates of individual cell types on 30-55% of the sections and by two points on

Table 3 Regional variation of cellularity and differential cell count in lymph nodes showing different histological patterns of diffuse Hodgkin's disease (cell counts were made with method A)

No. of serial section	Area marked on coverslip	Total No. of cells counted	No. of small graticule squares counted	Mean No. (and percentage) of cells of given type per small square within a graticule field						
				Total, all types	Lymphocyte	Plasma cell	Polymorphonuclear granulocyte	Normal mononuclear	Abnormal mononuclear	Abnormal multinuclear
Patient 1 — lymphocytic predominance										
1	A	1 671	20	83.6	81.1 (97)	0.05 (—)	0.2 (0.2)	1.75 (2.1)	0.4 (0.5)	0.1 (0.1)
4	B	10 288	121	85.0	82.2 (96.7)	0.02 (—)	0.26 (0.31)	2.33 (2.7)	0.13 (0.2)	0.09 (0.1)
	C	5 266	60	87.8	85.2 (97.1)	0 (0)	0.18 (0.2)	2.13 (2.4)	0.12 (0.1)	0.08 (0.1)
	D	1 865	20	93.2	91.0 (97.6)	0.1 (0.1)	0.15 (0.2)	1.65 (1.8)	0.2 (0.2)	0.1 (0.1)
	E	1 384	16	86.5	84.1 (97.2)	0 (0)	0.3 (0.4)	1.75 (2.0)	0.19 (0.2)	0.19 (0.2)
7	F	1 552	20	77.6	75.1 (96.8)	0 (0)	0.2 (0.2)	1.85 (2.4)	0.35 (0.4)	0.05 (0.1)
All counts				Mean SD	83.1 5.23	— —	0.215 0.055	1.91 0.264	0.232 0.117	— —
Patient 3 — mixed cellularity										
1	G	3 839	75	51.2	21.3 (41.7)	0 (0)	0.96 (1.8)	27.8 (54.3)	1.1 (2.1)	0.02 (—)
	H	2 816	60	46.9	16.9 (36.0)	0 (0)	0.35 (0.7)	28.9 (61.7)	0.7 (1.6)	0 (0)
4	I	4 800	117	41.0	20.1 (49.0)	0 (0)	2.46 (6.0)	15.3 (37.2)	3.2 (7.7)	0.04 (0.1)
	J	2 634	60	43.9	15.7 (35.8)	0 (0)	3.38 (7.7)	22.6 (51.4)	2.2 (5.0)	0.03 (0.1)
	K	2 863	60	47.7	16.3 (34.2)	0 (0)	1.72 (3.6)	28.5 (59.7)	1.2 (2.5)	0 (0)
	L	2 407	55	41.9	7.9 (19.3)	0 (0)	3.54 (8.7)	26.9 (66.4)	2.2 (5.5)	0.01 (0.03)
7	M	3 839	75	37.0	10.1 (27.2)	0 (0)	1.3 (3.5)	24.2 (65.6)	1.4 (3.7)	0 (0)
	N	2 816	60	49.5	20.6 (41.7)	0 (0)	0.5 (0.9)	28.0 (56.7)	0.4 (0.8)	0 (0)
All counts				Mean SD	16.1125 4.89	— —	1.78 1.24	25.28 4.61	1.55 0.92	— —
Patient 7 — lymphocytic depletion										
1	O	788	20	39.4	26.7 (67.9)	0 (0)	2.0 (4.9)	6.5 (16.4)	3.4 (8.5)	0.8 (2.00)
	P	762	20	38.1	28.7 (75.2)	0 (0)	0.7 (1.8)	4.5 (11.7)	3.8 (10.0)	0.5 (1.3)
	Q	740	20	37.0	27.1 (73.1)	0 (0)	1.2 (3.1)	4.6 (12.43)	3.6 (9.7)	0.6 (1.6)
	R	668	16	41.8	32.9 (78.9)	0 (0)	0.8 (1.9)	3.6 (8.7)	3.4 (8.2)	0.9 (2.2)
4	S	4 223	131	32.2	19.6 (61.8)	0.02 (0.05)	1.7 (5.2)	5.3 (16.4)	5.1 (15.7)	0.6 (1.75)
	T	1 715	47	36.5	21.4 (58.7)	0.02 (0.02)	2.3 (6.4)	6.6 (18.0)	5.6 (15.4)	0.5 (1.4)
	U	1 653	45	36.7	19.5 (53.1)	0 (0)	2.3 (6.2)	7.0 (19.1)	7.0 (19.1)	0.9 (2.5)
	V	2 364	50	47.3	31.2 (66.0)	0 (0)	2.2 (4.7)	8.4 (17.8)	4.5 (9.6)	0.8 (1.7)
7	W	524	16	32.8	20.7 (63.2)	0 (0)	1.3 (3.8)	5.7 (17.4)	4.8 (14.5)	0.4 (1.1)
	X	407	12	33.9	20.9 (61.7)	0 (0)	1.5 (4.4)	5.5 (16.2)	5.7 (16.7)	0.3 (1.0)
	Y	602	16	37.6	25.9 (68.8)	0 (0)	2.4 (6.5)	4.9 (13.1)	3.9 (10.3)	0.5 (1.3)
	Z	686	18	38.1	25.5 (66.9)	0 (0)	1.8 (4.8)	5.7 (14.9)	4.7 (12.2)	0.4 (1.2)
All counts				Mean SD	25.01 4.58	— —	1.68 0.37	5.69 1.29	4.63 1.10	0.60 0.20

2.4-15.9% of the sections. Whatever the imperfections of the method of sampling used in this present investigation, the results are much more meaningful than any that have been reported previously.

The primary aim of this investigation was to attempt to establish a quantitative cytological basis for distinguishing the diffuse subtypes of Hodgkin's disease as originally defined by Lukes

and Butler (1966) and later modified by Lukes *et al.* (1966). Our studies have confirmed most of the postulated diagnostic histological characteristics, namely, that lymphocytes are very numerous and Reed-Sternberg cells sparse in the lymphocytic predominance subtype, while abnormal cells are most numerous with relative depletion of all other types of cells in the lymphocytic depletion subtype. Lukes and Butler (1966) have claimed that the mixed cellularity subtype occupies an intermediate position between the other two subtypes of diffuse Hodgkin's disease. We cannot confirm this diagnostic criterion since we have found that lymphocytes were least numerous and normal mononuclear cells most frequent in this group. From our counts it is clear that the mixed cellularity subtype has positively distinctive characteristics, and it is not intermediate in cellular composition between the lymphocytic predominance and lymphocytic depletion subtypes. Furthermore, we have been unable to confirm in our cases of diffuse Hodgkin's disease that there is overall the inverse relationship between numbers of lymphocytes and Reed-Sternberg cells, emphasised so strongly by Lukes and Butler (1966). Lukes (1971) has subsequently stated that during the progression of the disease a dramatic increase

in the number of Reed-Sternberg cells may be seen before the lymphocytes become depleted; we do not know what proportion of the lesions are in this transitional stage. It is fitting, therefore, to ask ourselves why so many experienced histopathologists have accepted that lymphocytes are least numerous in the lymphocytic depletion subtype. Perhaps the explanation lies in the optical illusion illustrated in Figs 3 and 4. We believe that attention is distracted by the numerous pleomorphic cells in the lymphocytic depletion lymph node so that the frequency of lymphocytes is underestimated, while in the mixed cellularity node lymphocytes are more noticeable since attention is drawn less strongly by the normal and abnormal mononuclears which are the predominant cells.

Despite the discrepancies between our cell counts and the histological descriptions of the subtypes of diffuse Hodgkin's disease, it is reassuring that the lymph nodes originally selected by us as typical examples of the Rye subtypes can be shown to be distinguished by the relative frequency of lymphocytes, normal mononuclears, and abnormal mononuclear and multi-nuclear cells. Nevertheless there is considerable heterogeneity between our cases, and it is highly probable that, if less typical cases

Fig. 3 A lymphocytic depletion subtype of Hodgkin's disease. The lymph node was obtained at necropsy. This area contains 850 lymphocytes, 2 plasma cells, 5 polymorphonuclear granulocytes, 246 normal mononuclear, 190 abnormal mononuclear, and 33 abnormal multinuclear cells. (H and E $\times$ 250).

Fig. 4 A mixed cellularity subtype of Hodgkin's disease. The lymph node was obtained as a surgical specimen. This area contains 557 lymphocytes, no plasma cells, 4 polymorphonuclear granulocytes, 922 normal mononuclear, 50 abnormal mononuclear, and 6 abnormal multinuclear cells. (*H* and *E* $\times$ 250).

had been counted, more overlap would have been found. In this type of situation diagnostic classification will inevitably be arbitrary; consequently, in view of the inherent inaccuracy discussed above of assessment of numbers of cells during visual scanning of sections, it is hardly surprising that there is substantial failure of reproducibility in subtyping of Hodgkin's disease lesions by different experienced pathologists (Coppleson *et al.*, 1970, 1973; Correa *et al.*, 1973).

After empirical analysis of our counts in many different ways we found that the results from the three histological subtypes of diffuse Hodgkin's disease were most widely separated in three-dimensional plots of numbers of three different cell types per unit area. The chosen cell types were (a) abnormal mono- and multinuclears, (b) lymphocytes, and (c) normal mononuclears (Fig. 5). It may well be that this separation has no biological importance, but it is interesting to speculate whether the three parameters may reflect (1) tumour aggressiveness (relative numbers of abnormal cells), (2) specific immune response against neoplastic cells of cell-mediated type (relative numbers of lymphocytes), and (3) non-immunological stromal response of attempted repair or amplification of humoral

immunological reaction (relative numbers of normal mononuclears).

If our hypothesis is true it would indicate that in patients with lymphocytic predominance Hodgkin's disease there will be relatively low tumour aggressiveness and strong cell-mediated immunity so that it would hardly be surprising that such patients have a good prognosis. The hypothesis would further suggest that in lymphocytic depletion Hodgkin's disease the tumour aggressiveness will be high while there will be little immunological or non-specific response, so that it is hardly surprising that the prognosis for such patients is very poor. In mixed cellularity Hodgkin's disease the hypothesis indicates that the tumour aggressiveness is relatively low, but so also is the cell-mediated response. There is a considerable stromal reaction but this is probably relatively inefficient (compared with specific immunological reactions) in limiting the growth rate of the neoplastic cells. These factors may be related to the unpredictable but generally poor prognosis in this variant of the disease.

The technique we describe in this paper is so laborious that we cannot conceive of its ever being used in its present form in diagnostic pathology. We hope that the hypothesis presented in this

Fig. 5 (a) Three-dimensional representation of relative numbers of main cellular constituents in diffuse Hodgkin's disease showing characteristic clustering of findings in each of the three subtypes: $\blacktriangle$ = lymphocytic predominance; $\blacksquare$ = mixed cellularity; $\bullet$ = lymphocytic depletion. (b) The hypothesis presented in this paper attempts to relate the relative numbers of abnormal mono- and multinuclear cells to tumour aggressiveness, of lymphocytes to specific immunological reaction, and of normal mononuclears to non-specific stromal reaction.

paper may prove a basis for anticipating the survival of patients with diffuse Hodgkin's disease; consequently we are developing methods of using an image-analysing computer to assess the relative numbers of different types of cells in order to grade the lesions of Hodgkin's disease.

This study was supported by a grant to JSB from the Equipment Research Committee of the Scottish Home and Health Department. During part of this work AEL was financed by a student's vacation grant from the University of Dundee. We are grateful to Mrs R. Fawkes for preparing Figure 5.

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