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CELL PROLIFERATION OF CARCINOMA IN BILHARZIAL BLADDER: AN AUTORADIOGRAPHIC STUDY

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ABSTRACT

The rate of cell production in thirty-five cases of carcinoma in Bilharzial bladder was evaluated from the labelling index after *in vitro* incubation with [^{3}H]TdR. Squamous cell carcinoma was the most frequent histological type in this series and had a median LI of 8·0% which corresponds to a potential doubling time of 5·9 days. In squamous cell tumours the LI increased with the histological grade. Transitional cell tumours had a somewhat greater LI.

In all histological types the LI was significantly greater in the deep infiltrating parts of the tumour than in the superficial parts. The discrepancy between the estimated potential doubling time and the growth rate normally attributed to such tumours suggests the existence of an extensive cell loss factor. Areas of focal or diffuse mucosal hyperplasia were associated with increased LI.

INTRODUCTION

The association between bladder cancer and urinary Bilharziasis produces a distinct clinico-pathological pattern of disease. Well or moderately differentiated squamous cell carcinoma is the common histological variety as opposed to transitional cell carcinoma in Europe and North America. (El-Bolkainy *et al.*, 1972).

When first seen the majority of cases in Egypt have either invaded deep muscle or spread through the muscle wall (T_3) or invaded surrounding pelvic structures (T_4). Clinically these T_3 and T_4 cases exhibit a poor response to irradiation. (Awwad *et al.*, 1970). However, there is little experience on the radioresponsiveness of relatively small tumour burdens as might be present in T_1 or T_2 tumours or in gross or microscopic extensions that are likely to be found in the pelvis in a nominally T_3 tumour prior to surgery.

The immediate radiation response of a given tumor is likely to be influenced by its pre-treatment growth characteristics (Breur, 1966; Van Peperzeel, 1972; Malaise *et al.*, 1972;

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Tubiana *et al.*, 1975). In the present report the *in vitro* labelling properties of carcinoma arising in Bilharzial bladder is investigated in order to evaluate some of its growth parameters. Particular attention is focused on the labelling properties of those parts of the tumor infiltrating deep into the bladder wall or extending into the adjacent perivesical tissues since these are the parts that are likely to contribute to post-operative recurrence.

MATERIAL AND METHODS

Thirty-five patients with histologically confirmed carcinoma arising in a Bilharzial bladder were studied. The association with urinary Bilharziasis was confirmed on the basis of clinical, radiological and histological criteria (El-Bolkainy *et al.*, 1972). For histological typing and grading the World Health Organization classification of bladder tumours was adopted (Mostofi *et al.*, 1973).

All patients were clinically judged to belong to the T_3 category according to the UICC system of clinical staging (1974). The tumour volume varied between 22 and 102 cm^3 as evaluated by clinical, endoscopic and radiological procedures or measured directly in cystectomy specimens. In all patients the urine was infected, usually with *Escherichia coli.* Tumour samples were obtained from surgical cystectomy specimens in eleven cases and from multiple transurethral endoscopy biopsies in twenty-four cases. The specimens were processed immediately after excision. After cystectomy the bladder was opened aseptically and any superficial necrotic or loosely attached friable tissues were discarded. Approximately 0·5 cm^3 pieces were cut from each of the following parts of the tumour: superficial central, deep central, superficial marginal and deep marginal. Pieces taken from each region were processed separately. The deep samples included the outermost infiltrating margin of the tumour and any extensions into the perivesical tissue. Multiple specimens of bladder mucosa were obtained from different sites away from the tumour.

Endoscopic biopsy specimens were taken from the superficial and deep parts of the tumour and were also processed separately.

Each piece was cut into small fragments, approximately 1 mm^3, with two sharp scalpels. The fragments were then transferred to culture tubes containing 10 ml of medium 199 with 4·0 μCi/ml of [^{3}H]thymidine ([^{3}H]TdR) having a specific activity of 15 Ci/mM. The tubes were incubated at 37°C with agitation by continuous bubbling of a mixture of 95% O_2 and 5% CO_2 at a flow rate of 800 ml/min. A 4 hr incubation period was used in order to match the technique used by Hainau & Dombernowsky (1974) who investigated the *in vitro* labelling of transitional cell bladder cancer in Europeans.

At the end of the incubation period the fragments were washed in saline and fixed in Bouin's solution for 24 hr and then embedded in Paraplast wax, sectioned at 4 μm and dipped in Kodak NTB2 emulsion for autoradiography. The autoradiographs were exposed for 6 weeks and then developed in Kodak D 19b and Kodak 'Fixol'.

Scoring of labelled cells was restricted to the superficial 100 μm of each fragment, this being the depth where cell viability is maintained by adequate oxygen diffusion (Steel & Bensted, 1965). A cell was considered labelled if it had at least five grains above the background level. At least 2000 cells were scored for the determination of the labelling index (LI). An eye-piece graticule having an equivalent area of 100 μm^2 was used in order to facilitate counting.

RESULTS

For each tumour a mean LI was calculated for each of the superficial and deep parts since no appreciable differences were noted between the corresponding marginal or central regions. Tables 1 and 2 show the individual values of the LI together with the mean LI and standard error of the three histological types. The following points can be seen.

1. In all histological types and grades the LI was significantly greater in the deeper than in the superficial parts of the tumour. However such differences were less marked in undifferentiated tumours as shown in Table 1 for the three histological grades of squamous cell cancer.

TABLE 1. Individual LI values in squamous cell carcinomas of the bladder

Case no.	Superficial tumour	Deep tumour
	Grade I	
S.I.1	0·50	3·7
S.I.2	0·50	1·7
S.I.3	1·60	3·4
S.I.4	3·70	11·7
Mean ± s.e.m	1·6 ± 0·8	5·4 ± 2·2
	Grade II	
S.II.1	2·0	5·3
S.II.2	2·9	4·5
S.II.3	1·7	8·0
S.II.4	5·3	14·0
S.II.5	4·6	6·2
S.II.6	5·2	—
S.II.7	4·8	9·7
S.II.8	4·8	—
S.II.9	4·8	5·0
S.II.10	—	13·9
S.II.11	2·9	9·2
S.II.12	2·1	8·6
S.II.13	5·2	7·2
S.II.14	5·7	11·3
S.II.15	5·1	9·8
S.II.16	3·7	11·7
S.II.17	7·4	16·6
S.II.18	6·7	10·2
S.II.19	3·7	8·6
Mean ± s.e.m	4·4 ± 0·37	9·4 ± 0·82
	Grade III	
S.III.1	20·0	24·0
S.III.2	8·4	12·5
S.III.3	14·0	19·0
S.III.4	11·0	16·0
Mean ± s.e.m	13·4 ± 2·5	18·0 ± 6·5

TABLE 2. Individual LI values in transitional cell tumours and adenocarcinomas of the bladder

Transitional cell cancer Grade II			Adenocarcinoma Grade II		
Case no.	Superficial tumour	Deep tumour	Case. No.	Superficial tumour	Deep tumour
T.II.1	4·1	8·1	A.II.1	4·0	4·8
T.II.2	3·1	10·1	A.II.2	2·0	7·4
T.II.3	4·2	15·3	A.II.3	6·0	8·6
T.II.4	6·1	16·4			
T.II.5	3·0	10·3			
Mean ± s.e.m	4·1 ± 0·56	12·1 ± 1·6		4·0 ± 1·2	7·0 ± 1·1

TABLE 3. Labelling characteristics of hyperplastic bladder mucosa

Case	Nature of epithelium (no. of cell layers)	Distribution of label	LI Mucosa (%)		LI Deep tumour (%)
S.II.3	Stratified squamous (11 layers)	Mostly basal, few intermediate	Range median	2·0–6·8 3·0	8
S.III.4	Transitional (4–13 layers)	Mainly basal, few intermediate and superficial	Range median	0·0–6·2 3·0	16
S.II.13	Stratified squamous (12 layers)	Basal	Range median	2–4 3·7	7·2
S.II.4	Transitional (13–18 layers)	Mostly basal, few intermediate and superficial	Range median	0·6·2 4·4	14
A.II.2	Transitional (8–16 layers)	Basal	Range median	2–4·8 3·2	7·4

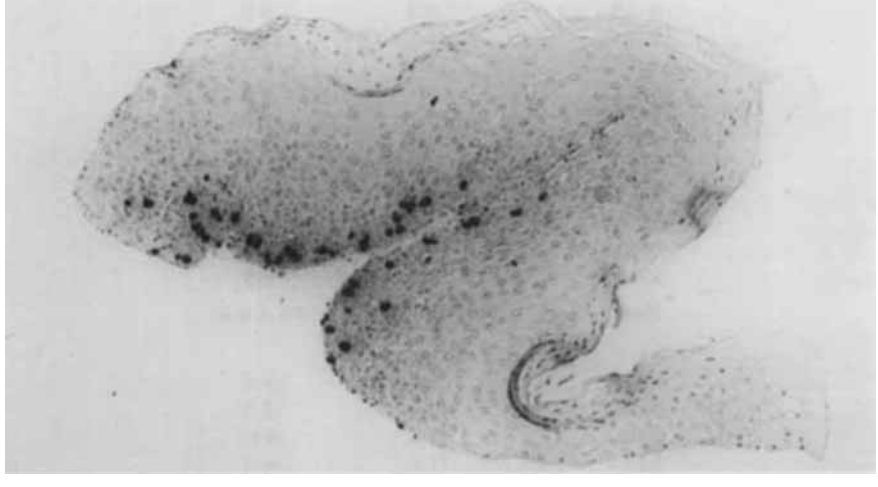

FIG. 1. Case S.II.3. Hyperplastic stratified squamous cell epithelium. Labelled cells are mostly found in the basal cell layer with few labelled intermediate cells. (Magnification ×50.)

2. The mean LI of the superficial tumour was remarkably similar in grade II of the three histological types. However the mean LI of the deep parts of five cases of grade II transitional cell carcinoma was significantly greater than the corresponding LI of grade II squamous cell cancer. Adenocarcinoma was found in three patients in this series, all belonging to grade II.

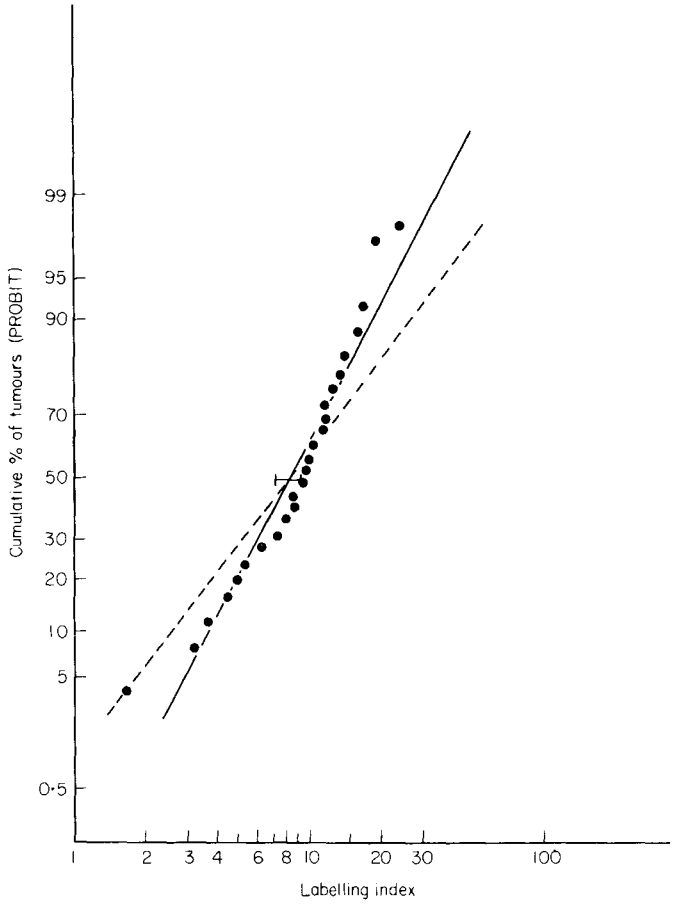

FIG. 2. The distribution of the individual LI values of the deeper tumour of twenty-five (——) cases of squamous cell carcinoma. The dotted line represents the log-normal distribution of sixty-eight (– – – –) cases of squamous cell carcinoma of different sites (Malaise *et al.*, 1973).

The mean LI of the deep parts seemed to be somewhat lower in comparison to the corresponding grade of the other two histological types.

3. In squamous cell carcinoma the LI increased with the histological grade with a tenfold difference in the LI of the superficial parts of the tumours between grades I and III and a threefold difference in the corresponding LIs of the deep parts. No significant correlation could be found between the tumour volume and the LI. In six out of the eleven cystectomy specimens the bladder mucosa did not show any appreciable hyperplastic changes. In these cases the LI varied between 0–1·0%. In the remaining five bladders epithelial hyperplastic

changes were histologically evident with marked regional variations. In these cases the LI of the bladder mucosa showed wide regional variations and ranged from 0% to 6·8% (Table 3). Hyperplastic regions composed of more than seven cell layers generally showed a relatively high LI. Labelled cells were mostly found in the basal cell layer but were also found in intermediate and even superficial cell layers (Fig. 1).

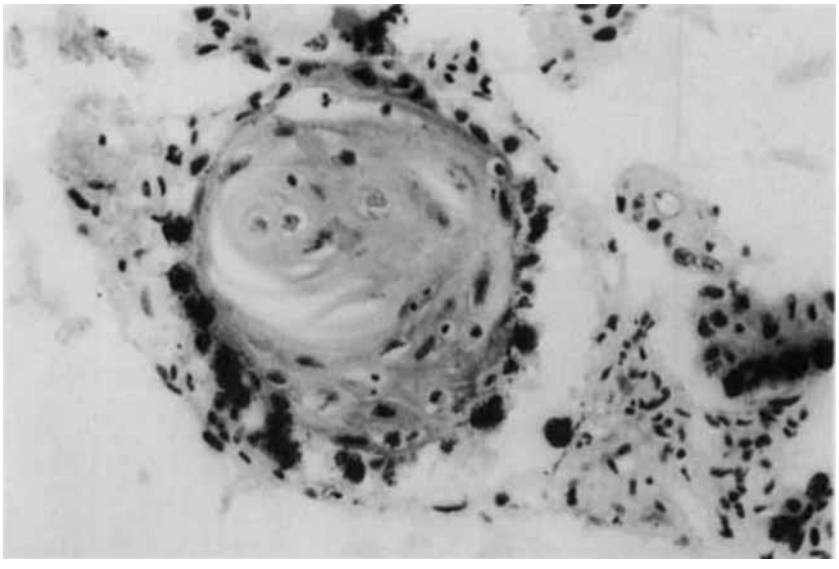

FIG. 3. A cell nest in a well differentiated squamous cell carcinoma. Labelled cells are found around the periphery of the nest. (Magnification ×700.)

DISCUSSION

Normal bladder mucosa is characterized by a slow rate of cell proliferation (Koss, 1961), and its Li *in vitro* does not exceed 1·0% (Hainau *et al.*, 1974). In the present series diffuse or focal epithelial hyperplasia induced by the chronic bladder infection was associated with increased cell proliferation and an elevation of the LI. It should be noted, however that the average LI of the bladder mucosa was always lower than that of the corresponding tumour (Table 3).

The present data indicate that carcinoma in Bilharzial bladder can have a high rate of cell production. Fig. 2 gives the distribution of the individual LI values of the deep part of the tumour of twenty-five cases of squamous cell carcinoma. The resulting regression line yields a median Li of 8·0% which corresponds to a potential doubling time (T_{pot}) of 5·9 days according to the relationship:

$$T_{pot} = \lambda\,(T_s/\mathrm{LI}),$$

where it is assumed that $T_s = 15$ hr and $\lambda = 0{\cdot}75$ (Steel, 1967).

These growth kinetics parameters are comparable to those of sixty-eight cases of squamous cell cancer of different sites that were collected from the literature and reviewed by Malaise *et al.* (1973) (Fig. 2). They are also comparable to the growth parameters of invasive

transitional cell bladder cancer in European patients (Hainau & Dombernowsky, 1974). The demonstration of a rising LI with increase of the histological grade is in accordance with the findings reported in case of non-Bilharzial bladder cancer (Fulker *et al.*, 1971, Hainau & Dombernowsky, 1974).

The demonstration in the present report of a greater LI in the deep infiltrating margin of the tumour is, most probably, a reflection of the existence of more favourable conditions e.g. a better vascular supply. Cell proliferation may be retarded in the more superficial layers by such factors as bacterial infection and deposition of Bilharzia ova which can produce chronic inflammatory reactions and ischaemia.

Clinical experience shows that the growth rate of cancer in Bilharzial bladder is much slower than that expected from the rate of cell production as estimated from the LI *in vitro*. This discrepancy could result from a high cell loss factor which characterizes a number of animal and human tumours (Denekamp, 1970; Malaise *et al.*, 1973). Cell loss through shedding into urine is an invariable finding in this form of cancer and the majority of patients suffer also from gross necroturia. Moreover the squamous cell variety has a strong tendency for differentiation that may proceed to keratinization. This can contribute to a slower clinical growth rate since differentiated non-cycling elements may remain for some time within the tumour volume before they are finally lost (Fig. 3).

The clinical significance of the growth characteristics of carcinoma in Bilharzial bladder and their correlation with its response to irradiation and long term treatment end-results are currently under study.

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