Glycolytic Enzyme Activity in Bladder Tumours

A. J. CARR, Senior Lecturer in Pathology, University of Aberdeen AND J. H. STEYN, Consultant Urologist, Aberdeen Royal Infirmary, Aberdeen, Scotland

SUMMARY

The activity of β-glucuronidase and β-N-glucosaminidase was studied in normal bladder tissue, benign bladder papilloma and in bladder carcinoma. The enzyme activity was measured spectrophotometrically and a comparison made of the activity of the different grades of tumour. Tumour tissue has a higher activity than normal bladder tissue and maximal activity is found in well differentiated malignant tumour.

Fishman and Anlyan found that malignant tissue and tissue in the process of regeneration have a richer content of β-glucuronidase than stable normal tissues. Boyland et al. showed that the urine of patients with bladder cancer contained an increased amount of β-glucuronidase. These findings aroused considerable interest particularly among the Italian workers and were subsequently confirmed by Mattea and Parazzolo and Pavone-Macaluso.

Considerable speculation arose as to the possible role of this enzyme in the development of bladder tumours. It has also been found that the serum β-glucuronidase activity of patients with bladder tumours may be raised. Boyland et al. and Mattea suggested that the urinary glucuronidase activity is derived from the increased serum levels.

Hradec et al. found no correlation between the level of the enzyme and the degree of malignancy, and that removal of the neoplastic tissue did not give rise to a decreased enzyme excretion, suggesting that the neoplastic tissue was not the source of the enzyme. On the other hand Appert and Richterich, studying a group of dye-workers, showed that with removal of the tumour the glucuronidase activity decreased, thereby suggesting that the tumour itself was the source of the β-glucuronidase activity.

The purpose of this article is to present a preliminary report on a study undertaken by us to assess the level of glycolytic enzyme activity in a variety of bladder tumours. Apart from β-glucuronidase activity, the β-N-acetyl glucosaminidase activity was also studied as the latter is a glycosidase related to glucuronidase and because the two enzymes, apart from similar activities, also share similar biological distribution patterns.

TECHNIQUE

Fresh samples of bladder tumour or mucosa were obtained by cystoscopic biopsy or from cystectomy specimens and selected to exclude necrotic tissue as far as possible.

Within minutes, all specimens were 'snap-frozen' on a metal chuck with CO₂ gas and then sectioned at −15°C on the Slew Cryostat. Sections were taken at intervals and stained with haematoxylin and eosin for histological examination. Others were collected, weighed and homogenized with iced water in Teflon glass homogenizers and the volume adjusted to 5 ml. This results in the release of coloured aglycone in amounts proportional to the enzyme activity.

Estimation of β-glucuronidase activity was carried out by incubation of 0.5 ml aliquots of the homogenates in 0.5M acetate/sodium hydroxide buffer at pH 4.5 using phenolphthalein glucuronide substrate in a final concentration of 0.00125M in the incubation mixture. After incubation for 1 hour strongly alkaline buffer was added to stop the reaction and the liberated phenolphthalein was measured using a 'Unicam' SP 500 spectrophotometer at 545 A wavelength.

The substrate p-nitrophenyl-β-N-acetyl-β-D-glucosaminide was used to estimate the β-N-acetyl glucosaminidase activity by incubation for 1 hour with 0.5 ml aliquots of the homogenates in 0.5M citric acid NaOH buffer containing NaCl at pH 4.3. The liberated p-nitrophenol was measured on the spectrophotometer at 430 A wavelength.

By calibration the activities of each enzyme were expressed as micrograms of aglycone released per gram (wet weight) of tissue assayed per hour of incubation.

The haematoxyline and eosin sections were assessed from two standpoints:

A. Cytology with grading of the tumour into: + benign papilloma; ++ well-differentiated carcinoma; +++ moderately differentiated carcinoma; and ++++ undifferentiated or anaplastic carcinoma. No specific attention was paid to 'invasion'.

B. Cell content so that allowance could be made for undue amounts of connective tissue present in the different specimens.

RESULTS

Forty-five separate estimations were made. The glucuronidase activity is shown in Fig. 1 and the acetyl glucosaminidase activity is shown in Fig. 2. In each instance,
the enzyme activity is plotted against the cytological pattern of the tumour. There is considerable grouping of the enzyme activity of the different grades of tumour, but occasional values both for glucuronidase activity and for acetyl glucosaminidase activity are very high. When the

mean values for each group are taken and plotted graphically, the distribution seen in Fig. 3 is obtained. The values for normal bladder mucosa are low. The values for the benign papilloma are much higher than for normal tissue, rising even higher with the change to a well-differentiated malignant tumour. However, as the degree of malignancy increases, the values drop down to almost the same level as those of the benign papilloma for the anaplastic tumours.

**DISCUSSION**

Willis, in discussing the invasive properties of tumour cells, felt they were related to:

1. The power of progressive multiplication which may act through pressure gradients as described by Young. Although this may be important, it is not a vital aspect of invasion.

2. Motility of the individual cancer cells, although possibly occurring in mesenchymal tumours, is unlikely to play any part in epithelial tumours.

3. Metabolites and enzymes capable of affecting the surrounding tissues are undoubtedly produced by a tumour. A number of workers have shown that some tumours produce an excess of enzymes capable of altering connective tissue ground substance and hence play a part in invasiveness.

4. Loss of growth restraint by the normal cells is unlikely to be a factor as transplanted malignant cells can cause invasion, whereas transplanted normal cells failed to do so.
rapidity of growth. This method of spread obtains in 70% of malignant bladder tumours.

2. The tentacular spread, where the malignant cells grow between the individual muscle cells and which is said to be related to the grade of differentiation of the tumour. This occurs in 27% of tumours.

3. Lymphatic permeation which occurs in only 3% of tumours. Incidentally, the majority of bladder lymphatics lie near the surface of the bladder and in deeply invasive tumours, the amount of lymphatic involvement rises steeply.

The spread of bladder cancer is a complex mechanism. There is the *en bloc* invasion largely as a result of progressive multiplication and the tentacular spread due to anaplastic cells invading the muscular layer. Then there are the anomalous findings such as the better-differentiated cells appearing in metastatic deposits. Is this a manifestation of enzyme activity in that it is the better-differentiated cells which are able to produce enzymes in greater quantities?

When the present study was initially undertaken, it was anticipated that with increasing malignancy greater quantities of enzyme would be produced, but as shown in Fig. 3, there is a much higher production of enzyme by tumour cells than by normal tissue. However, once the differentiation becomes more marked, the quantity of enzyme production starts falling, which in retrospect is a reasonable development.

A correlation of the enzyme patterns with the clinical behaviour of the tumours has been totally unrewarding. It has not been possible to predict on the basis of enzyme activity, how the tumour would behave clinically. However, it is interesting to note that when a particularly high β-N-glucosaminidase activity was present, as was found in a few of the tumours in the moderately differentiated or well-differentiated carcinomas, the clinical presentation was that of a very bulky tumour.

Tumour behaviour is an extremely complex phenomenon and in a preliminary study of this nature it is only possible to hint at a possible factor in the invasive behaviour of bladder cancer. The main spread of bladder tumours is by *en bloc* invasion and to a lesser extent by tentacular spread, but it may be that the glycolytic enzyme activity is related to the unexpected findings of metastatic spread. Further study is required to elucidate this problem.

REFERENCES