An investigation into the enzyme histochemistry of adenocarcinomas of human large intestine and of the transitional epithelium immediately adjacent to them

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SUMMARY Histochemical enzymatic studies were performed on 30 freshly resected large bowel carcinomas, 30 samples of normal colonic epithelium, and six samples of the histologically normal epithelium (so-called transitional epithelium) immediately adjacent to a carcinoma. Five enzymes were studied: nicotine adenine dinucleotide tetrazolium reductase (NADH-TR), glucose-6-phosphate dehydrogenase, succinate dehydrogenase, monoamine oxidase, and acid phosphatase.

Quantitative and qualitative differences in enzyme activity were observed between normal, transitional, and carcinomatous mucosa as follows: monoamine oxidase activity was moderate in normal mucosa, high in transitional mucosa, and low in carcinoma. Succinate dehydrogenase activity was high in transitional mucosa and low or moderate in normal and carcinomatous mucosa. Glucose-6-phosphate dehydrogenase activity showed a gradation from low in normal mucosa to high in carcinoma while acid phosphatase showed the reverse of this pattern. The tetrazolium reductase activity was low or moderate in normal and transitional mucosa and high in carcinoma.

These differences in enzyme activity and their possible clinical and metabolic significance are discussed.

During the course of an investigation into the enzyme histochemical patterns of normal colon, colonic adenocarcinoma, and carcinomatous metastases to mesenteric lymph nodes, the mucosa immediately adjacent to a carcinoma, which is morphologically normal, was observed to show characteristic enzymatic changes which are quantitatively different from those in the carcinoma and the more distant normal mucosa. Various workers (Wattenberg, 1959a and b; Nachlas and Hannibal, 1961; Mori, Sugimura, Matsumura, and Kawashima, 1963; Cohen, Elizalde, and Miller, 1968; McGinty, Delides, and Harrison, 1973) have studied enzyme patterns in colonic adenocarcinoma but only Filipe (1971) has described changes in enzyme patterns and alterations in mucosubstances (Filipe, 1969) in what she calls 'transitional' epithelium. We feel that the findings we describe may have clinical significance in the assessment of precancerous lesions.

Materials and Methods

Thirty fresh surgically resected adenocarcinomas of large bowel, each of which had sufficient attached normal colon to allow a study of normal mucosa at a distance of more than 10 cm from the carcinoma, were studied. In six of these a special study was made of the histologically normal 'transitional epithelium' immediately adjacent to the carcinoma. All blocks of tissue were snap frozen in liquid nitrogen immediately following resection, and stored at minus 30°C for a maximum of two days.

The enzymes demonstrated histochemically and the techniques used were: acid phosphatase (Barka and Anderson, 1962), monoamine oxidase (Glenner, Burtner, and Brown, 1957), succinate dehydrogenase, (Pearse, 1972), tetrazolium reductase (Pearse, 1972), and glucose-6-phosphate dehydrogenase (Pearse, 1972).

Frozen serial sections of unfixed tissue were cut at a thickness of 12 µ, and were mounted on coverslips. Those for haematoxylin and eosin (H and E) staining and for acid phosphatase demonstration

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were postfix in 10% formal calcium at 4°C. Two sections were used for the substrate control when this was necessary, and two were used for the test itself. The first two and the last two sections of each tissue block were stained with haematoxylin and eosin and examined before commencing any enzyme work to ensure that the sections contained the required tissue and were correctly orientated. The enzymes were quantitated visually according to their depth of staining, using an ordinary light microscope at a magnification of ×20, and a quantifying scale of high, moderate, or low. Mean values were calculated for normal, transitional, and carcinomatous mucosa.

Results

Intracellular enzyme activity was localized to the basal portion of the cytoplasm in normal absorptive and goblet cells except in the case of glucose-6-phosphate dehydrogenase where the location was both apical and basal. In transitional mucosa the intracellular location was essentially similar although succinate dehydrogenase and monoamine oxidase activity showed a more diffuse localization in three patients. By contrast in carcinomatous epithelium all enzymes were either diffusely localized or in the apical region of the carcinoma cells.

Details of staining intensity in normal, transitional, and carcinomatous epithelium for each of the five enzymes studied are shown in histogram form in fig 1 and examples are given pictorially for two enzymes in figures 2 and 3. No further description appears necessary.

Discussion

In this study we have equated enzyme activity with depth of staining, which has been estimated on a purely visual basis. Visual assessment can be highly subjective, and this equation is only justified when techniques are standardized: one person does the assessing, sections are of uniform thickness, and concentration of final reaction product is directly related to enzyme concentration. More accurate quantitation, however, is difficult and time consuming and does not lend itself to clinical problems requiring a rapid solution. It is also inaccurate, though convenient, to talk about enzyme 'staining'. The staining represents a coloured insoluble reaction product which is the result of enzymic activity; one cannot stain an enzyme directly. We have not attempted to classify fine distinctions in staining density but we are satisfied that our results are at least reproducible and our three categories visually separable.

An increase in tetrazolium reductase activity in adenocarcinoma as compared with normal colon has been recorded by Wattenberg (1959a), Nachlas and Hannibal (1961), and McGinty and colleagues (1973) as well as by ourselves. Filipe (1971) records uniformly high levels in normal, transitional, and carcinomatous mucosa while we found only moderate activity in normal and transitional mucosa.

The findings of Mori et al (1963), Cohen et al (1968), and Filipe (1971) of moderate activity of glucose-6-phosphate dehydrogenase in adenocarcinoma of the colon, with low activity in normal mucosa, agree with our findings, although McGinty et al (1973) found moderate activity in normal mucosa, with high activity in carcinoma. Filipe (1971) found high activity in transitional mucosa whereas our results showed only a slight increase compared with normal.

Wattenberg (1959a and b) and McGinty et al (1973) found much less activity for succinate dehydro-
Fig 2  Technique for monoamine oxidase. Transitional mucosa containing a high concentration of enzyme as judged by final reaction product on the right, carcinomatous epithelium with low enzyme concentration on the left. × 30.

Fig 3  Technique for succinate dehydrogenase. Transitional mucosa showing a high concentration of enzyme as judged by final reaction product in the upper part of the field, carcinomatous epithelium with low enzyme concentration in the lower part. × 30.
genase in carcinoma than in normal mucosa, which was found to show moderate activity. Czernobilsky and Tsou (1968) found a generally higher activity in adenocarcinoma. Filipe (1971) recorded low to moderate activity in the normal mucosa, with a slight increase in transitional mucosa and moderate activity in the tumour. These results do not agree with our finding of low to moderate activity in the normal and cancerous crypt cells, with marked activity in transitional mucosa.

Low to moderate monoamine oxidase activities in normal crypt cells, similar to our results, were recorded by Wattenberg (1959a). However, this author also found a relative increase in activity in the well differentiated carcinomas and a relative decrease in the poorly differentiated ones. McGinty et al (1973) recorded strong activity in normal and moderate activity in adenocarcinoma, qualitatively similar to our results of moderate activity in normal and low activity in the tumour. Filipe's work did not include monoamine oxidase, and therefore our finding of marked activity in transitional mucosa is impossible to compare.

Our observation that adenocarcinoma of colon only has slight acid phosphatase activity when compared with the moderate activity found in normal crypt cells corresponds well with the work of Filipe (1969) and of Czernobilsky and Tsou (1968). McGinty et al (1973) found marked acid phosphatase activity in normal mucosa, with a marked reduction in adenocarcinoma.

The clinical significance of these findings lies in the fact that histochemical enzyme techniques can demonstrate distinctive intracellular changes before these become observable histologically. Wattenberg (1959a and b), in his studies on proliferative lesions of the large intestine, recorded qualitative and quantitative changes in succinate dehydrogenase and cytochrome oxidase in atypical glands found near malignant polyps and other benign proliferative lesions. The author thought that these changes might be an early indication of carcinogenesis. Further unpublished work by Filipe (1969) has documented a disruption of intracellular enzyme localization for glucose-6-phosphate dehydrogenase, tetrazolium reductase, and succinate dehydrogenase in crypt cells adjacent to tumours. Enzyme changes of this nature could be responsible for the failure of sulphation of mucopolysaccharides that Filipe noticed in transitional mucosa (Filipe, 1969).

We feel that these enzyme changes are likely to be consequent on, rather than the cause of, carcinogenesis. Their basis may lie in an intracellular metabolic and/or genetic change in enzyme control, may depend on extracellular environmental factors, or may merely represent a normal cellular adaptive response. The high levels of succinate dehydrogenase exemplify this last idea. A reduction in blood supply due to mechanical compression by a tumour mass, or due to changes in connective tissue and blood vessel wall mucopolysaccharides, as described near tumours by Majewski, Tkaczyk, and Majewski (1966), would decrease the oxygen and substrate supply to the transitional mucosa. A compensatory enzyme 'hyperplasia' of the Kreb's cycle enzymes might enable the cell to use at least as much substrate or oxygen as it received. The fact that the levels of these enzymes subsequently fall to those levels found in adenocarcinoma of the colon can be explained on the basis of the cells having acquired alternative pathways of metabolism more suited to their requirements than a long-term massive increase in enzyme protein.

The increase in succinate dehydrogenase implies relatively normal functioning of the electron transport chain, an implication consistent with the finding of identical levels of activity for tetrazolium reductase in both normal and transitional mucosa. The cells in the carcinomas are thought, on the basis of their high tetrazolium reductase levels, to be dependent on diaphorase activity as a 'sink' for metabolic hydrogen that would normally go through the electron transport chain to oxygen. The cells of the transitional mucosa appear not to have developed dependence on the diaphorases in this way. The elevation in glucose-6-phosphate dehydrogenase could be explained on the basis of the cell requiring more riboses for nucleic acid synthesis if it is beginning to develop the increased mitotic capacity of a cancer cell.

The changes observed for monoamine oxidase and acid phosphatase in transitional mucosa are difficult to interpret metabolically. Monoamine oxidase and succinate dehydrogenase, the two enzymes which show a marked rise, are also the two enzymes for which a change of localization in transitional cells was observed.

Certain conclusions can be drawn from this study. There are well marked quantitative differences, in the five enzymes tested, between normal and carcinoma-tous colon. These differences are variable in their extent. Qualitative changes are limited to small differences in localization of the enzyme between normal and carcinomatous mucosa. All the enzymes, except tetrazolium reductase, show differences of 'staining' intensity in transitional mucosa from the pattern obtained for normal or cancerous mucosa. This could be a manifestation of developing cancer cell metabolism or of normal cellular adaptation to a changed environment. We feel that a combination of enzyme techniques and techniques for mucobases as recommended by Filipe (1969) could be of
An investigation into the enzyme histochemistry of adenocarcinomas of human large intestine

value in detecting malignant transformation in benign lesions, or in detecting precancerous change in histologically normal mucosa in a preselected, vulnerable population. Their use should be explored in familial adenomatosi, chronic ulcerative colitis, and histologically benign adenomas and papillomas of large intestine.

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