A Histochemical Study of Succinic Dehydrogenase and Cytochrome Oxidase in Proliferative Lesions of the Large Intestine*

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Morphologic histochemical studies of a number of oxidative enzymes in normal mucosa and proliferative lesions of the large intestine of man have previously been reported (15). In these studies, a weak succinic dehydrogenase activity was observed in poorly differentiated carcinoma of the large bowel. This finding is in accord with other investigations in which a low succinic dehydrogenase activity has been found in a variety of malignant neoplasms (11, 12). However, since a decrease in activity of this enzyme also occurs in rapidly proliferating nonmalignant tissue, such as regenerating liver (7, 8, 14), it is apparent that the metabolic change it reflects is not specific for malignancy.

In contrast to the low succinic dehydrogenase activity of poorly differentiated carcinoma of the large intestine, epithelial components with high as well as low activities of this enzyme were found to be present in well differentiated malignant glands of this anatomic site (15). Similarly, in benign proliferative lesions of the large bowel, epithelia with quite marked differences in succinic dehydrogenase activity were observed in specific components of these tissues. In some instances, the weakly reacting benign elements showed as little or even less activity than that found in malignant tissue.

In the present report, the results of further investigations of succinic dehydrogenase activity in proliferative lesions of the large intestine are presented. It will be shown that there is a decrease in the activity of this enzyme in elements of all proliferative lesions studied. This decrease in enzyme activity would appear to reflect an aspect of altered metabolism common to both benign and malignant proliferative processes of the mucosa of the large intestine. With regard to the observation of epithelium manifesting relatively high succinic dehydrogenase activity, it will be shown that in benign proliferative lesions of the large intestine a well defined orientation exists between the cells with differing activities. The epithelium with weak activity is found to be superficial to the epithelium manifesting a more intense reaction in the glands of these lesions. In malignant tissues this relationship is lost, and this loss may constitute an early indication of carcinogenesis.

In addition to succinic dehydrogenase, histochemical studies of the enzyme cytochrome oxidase have likewise been carried out in proliferative lesions of the large bowel. The activity of this enzyme also decreases in elements of all the proliferative lesions studied. However, its activity frequently does not parallel that of succinic dehydrogenase.

MATERIALS AND METHODS

The tissues employed in the present study were obtained from surgically resected specimens. Included are 61 carcinomas of the large intestine, 60 adenomatous polyps, and 29 areas of focal hyperplasia. The lesions of focal hyperplasia were all present in the mucosa close to infiltrating carcinoma. Succinic dehydrogenase activity was studied in all the tissues enumerated. The procedure used for cytochrome oxidase has only recently become available (6) and was employed in tissues obtained during the latter part of the study: carcinoma, 20; adenomatous polyps, 25; and hyperplasia, 12.

The procedures for the handling of the tissues and for the histochemical demonstration of succinic dehydrogenase, DPN diaphorase, and TPN diaphorase have been described previously (15). Cytochrome oxidase was demonstrated by a modification of the Nadi reaction† (6). In addition to the 4-amino-1-N,N-dimethylamidopylamine used initially in this study was generously made available to us by Dr.

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the use of untreated frozen sections for the modified Nadi reaction, parallel sections extracted with ethyl acetate for 5 minutes at 4°C were employed. This extraction did not significantly alter the enzyme activity, but the removal of lipide gave a somewhat sharper localization of the colored deposition product. A 10-minute incubation period at 37°C was used for the succinic dehydrogenase, DPN diaphorase, and TPN diaphorase procedures, and a 15-minute incubation period for the cytochrome oxidase procedure. Sections 16μ thick were employed for all the histochemical work.

RESULTS

Normal mucosa.—In the normal mucosa, the histochemical reactions differ in the surface epithelium, the nonmucoid epithelium at the base of the glandular crypts, and the mucoid epithelium lining the remainder of these crypts. This mucoid epithelium is characterized by the presence of large numbers of goblet cells. With the succinic dehydrogenase procedure, the nonmucoid cells at the base of the crypts give the most intense reaction, which is manifested as a dark blue staining of the cytoplasm of these cells. The staining is not uniform but is most pronounced in the periluminal area of the cytoplasm. The surface epithelium stains a slightly lighter color than the basal cells. However, in the surface epithelium the greatest color intensity is at the base of the cells. The least intense staining occurs in the mucoid cells of the crypts (Fig. 1). The histochemical procedure for cytochrome oxidase results in a slightly lighter staining of all the epithelial components of the mucosa than that observed for succinic dehydrogenase. The surface epithelium and all the crypt epithelium stains with about the same intensity as the nonmucoid basal cells. The deepest staining is in the periluminal area. With the procedure for demonstrating cytochrome oxidase, very light and often no staining at all occurs in the surface epithelium, and in all of the crypt epithelium including the nonmucoid basal cells.

Benign adenomatous polyps.—Benign adenomatous polyps are quite variable both in their morphologic features as well as in their histochemical reactions. In most of these lesions, however, three distinctive morphologic components are apparent: (a) the surface epithelium, (b) atypical glands composed of nonmucoid cells or cells having smaller mucus droplets than those of the crypt epithelium of the normal mucosa, and (c) glandular elements very closely resembling those of the normal colon. The atypical glands frequently occupy the superficial portion of the polyp. The depth to which they penetrate and the relative portion of the total glandular mass of the polyp which they comprise vary.

The surface epithelium and outer portion of the crypts of the atypical glands stain lightly with both histochemical procedures (Figs. 4, 5). In some instances, no staining at all is observed; in other cases, the staining may be only slightly lighter than that of the surface epithelium of the normal mucosa. The deeper lying atypical epithelium generally results in an intensity of staining of the deeper lying atypical epithelium which is comparable to or at most slightly greater than that of the crypt epithelium of the normal mucosa (Fig. 5).

Some of the glandular elements of adenomatous polyps resemble those of the normal mucosa both in morphologic form and histochemical reactions (Figs. 6, 7). Goblet cells containing mucoid epithelium similar to that found in the crypts of the normal mucosa comprise most of this tissue. In the terminal glandular elements adjacent to the stalk of the polyp, nonmucoid epithelium is found which resembles that of the nonmucoid basal cells of nor-

Marvin M. Nachlas, Dept. of Surgery, Johns Hopkins Medical School; subsequently, preparations synthesized by Mr. J. Lionel Leong, Dept. of Pathology, University of Minnesota, were employed.
for each gland form the more intense staining oc-
curred in the deeper portion and weaker activities
In adenomatous polyps, because of the multi-
plely differentiated malignant gland forms. However, a careful study indicates that
for each gland form the more intense staining oc-
curred in the deeper portion and weaker activities
are present more superficially. When a polyp has
essentially a double layer of glands with elements
of the atypical glands adjacent to the bowel lumen
and glands resembling those of the normal mucosa
in a deeper position, there is a corresponding
doctrine of lighter and more deeply staining
gland components evident with the succinic de-
hydrogenase procedure.

**Infiltrating carcinoma.**—Most carcinomas of the
large intestine are composed largely of well differ-
entiated malignant gland forms. However, even in
such well differentiated lesions, some poorly differ-
entiated elements occur, and occasionally these predominate. With both the succinic dehydro-
genase and cytochrome oxidase procedures, poorly differentiated carcinoma stains weakly or not at
all. In well differentiated carcinomatous glands, a
more complex situation exists, particularly with regard to succinic dehydrogenase. While much of the epithelium stains very lightly, some compo-
nents show relatively intense staining, and in some
instances deeply stained tumor cells are present
(Fig. 8).

While the cytochrome oxidase activity of car-
cinomas shows some variation, the vast bulk of this
differentiated tumor tissue stains very weakly for the
enzyme.

**In situ carcinoma.**—*In situ* carcinoma can be
found in an occasional adenomatous polyp.
Twelve such lesions were available for study in the
present work. The malignant areas are charac-
terized by disorganization of the epithelium, with
loss of nuclear polarity. Irregularity of nuclear size
with large and bizarre forms may be present, and
enlarged nucleoli may also be seen. In some in-
stances, intraglandular bridging and a cribriform
pattern are found. Where manifestly malignant
morphologic features occur, the histochemical re-
actions are similar to those observed in infiltrating
carcinoma. In many areas of the malignant polyps
the epithelium of the atypical glands present does
not show morphologic evidence of malignancy.

However, a decrease in succinic dehydrogenase
and cytochrome oxidase activity may occur in
such epithelium, including that portion occupying
the deepest part of the atypical glands (Fig. 9). It
is apparent that in these atypical glands, there has
been a disruption in the orientation characteristic
of benign proliferative lesions in which cells with
relatively high succinic dehydrogenase activity
occupy basal gland areas.

In malignant polyps manifesting a loss of orient-
ation of gland elements with differing succinic
dehydrogenase activities, the decrease in succinic
dehydrogenase activity which is observed in the
deep portion of the atypical glands may be uniform
or there may be an irregular decrease in activ-
ity imparting a "moth-eaten" appearance to the
glands (Figs. 9, 12, and 14). In some of these
areas, transitions can be observed between epit-
elium which appears benign as judged by classi-
cal morphologic features and adjacent manifestly
malignant epithelium, and in which both the be-
going and malignant-appearing morphologic forms
have low activities of the two enzymes (Figs. 11–
14). These findings suggest that the morphologi-
cally benign-appearing epithelium has undergone
some of the metabolic changes found in carcinoma.
Additional evidence for this interpretation is the
frequent occurrence of high DPN diaphorase activity
in such epithelium (Fig. 10). Occasionally,
an increase in TPN diaphorase will also be ob-
served. A high DPN diaphorase and TPN dia-
phorase activity have previously been described as
being part of the histochemical reaction pattern
characteristic of carcinoma of the large bowel (15).

**DISCUSSION**

In the present study, morphologic histochemical
techniques have been employed in an effort to delineate enzymatic characteristics of the diverse cell
types found in the normal mucosa and in prolifer-
ating lesions of the large bowel. Since in considering
the results obtained, implications are drawn as to
the relative activities of each of the enzymes in the
cells under investigation, it is pertinent to discuss
initially the validity of making such semiquanti-
tative interpretations.

The histochemical procedures for demonstrat-
ing succinic dehydrogenase and cytochrome ox-
dase are based upon quantitative reactions. In the
case of succinic dehydrogenase, this has been
shown to be true for homogenates (9) and also for
tissue sections prepared in a manner comparable
to that used in the present study (3, 4). Freezing
and thawing do not appreciably alter the enzyme
activity as determined by tetrazolium salt reduc-
tion (9). For the modified Nadi reaction, a quanti-
tative adaptation of the histochemical procedure comparable to that developed for the original Nadi reaction (13) has not been reported. However, it has been found that the deposition product can be quantitatively extracted from tissue sections with a mixture of equal parts of tetrachloroethylene and ethanol\(^2\) following procedures previously described for this type of extraction (3, 4). Quantitative studies of the amount of deposition product formed with variation of conditions such as the duration of incubation, the amount of tissue present, and the cytochrome c content of the reaction mixture indicate that the modified Nadi reaction in tissue sections is of a quantitative nature.\(^3\)

In the two morphologic histochemical procedures a crude approximation of the relative enzyme activities of various cells has been made from a visual estimation of the amount of deposition product present. Such estimations can lead to erroneous interpretations if they are arrived at uncritically. In particular, variations in the size and distribution of the deposition products of cells whose activities are being compared can lead to difficulties in interpretation (2). This point has been kept in mind in reporting the relative enzyme activities from the histochemical preparations used in the present investigation. The decreases in enzyme activity which have been emphasized in the descriptions of proliferative lesions are of such a nature that it is not possible for them to be due to a peculiarity of the form of the deposition product. In many instances the cells are either completely colorless or at most have only a trace of color. Likewise, in those instances in which increases in staining intensity are alluded to, the distribution of the deposition product and the depth of the color are such as to make it very unlikely that a misinterpretation is being made.

In elements of all the proliferative lesions of the large bowel studied, a decrease in staining for succinic dehydrogenase and cytochrome oxidase as compared with that found in the normal mucosa has been observed. In malignant lesions, a weak reaction for both succinic dehydrogenase and cytochrome oxidase is found in poorly differentiated tumor epithelium. In benign and well differentiated malignant proliferative lesions, components with low activities of the two enzymes are also present. These include the surface epithelium and outer portion of the crypts of atypical glands of benign adenomatous polyps, the surface epithelium and all the crypt epithelium except for the nonmucoid basal cells of hyperplastic mucosa, and some glandular elements of well differentiated carcinoma.

In contrast to the glandular components of benign proliferative lesions which have low succinic dehydrogenase activity, other components are present which have high activity. These elements with the high activity occur in the basal portion of glandular crypts. There is thus a well defined orientation of cells with low and high succinic dehydrogenase activity, the weakly reactive components being superficial to the more strongly reactive ones. A characteristic feature of the high succinic dehydrogenase activity of the basal elements is that it is not associated with a comparably high cytochrome oxidase activity and in this regard parallels the findings in the basal cells of the glands of the normal mucosa. This discrepancy in the level of activities of the two enzymes relative to one another suggests the possibility that more than one relationship may exist between succinate oxidation and electron transport. This concept is in accord with recently reported quantitative studies of succinate oxidation by beef heart mitochondria which also indicate that more than one electron transport system occurs (1). Since a decrease in succinic dehydrogenase activity has been found to reflect a metabolic alteration accompanying cellular proliferation in the mucosa of the large intestine, the occurrence of more than one relationship between succinate oxidation and enzymes involved in electron transport would suggest that more than one factor affecting succinate oxidation may operate in the control of cellular proliferation.

The cells in which the high succinic dehydrogenase is accompanied by a relatively weak cytochrome oxidase activity are those which are most strategically placed for control of gland penetration. This is clearly apparent for the cells at the base of glandular crypts which define the position of the base of the glands and must therefore be in some sort of equilibrium with the underlying tissues. It thus appears possible that the form of succinic dehydrogenase which is accompanied by a relatively weak cytochrome oxidase activity reflects a special mechanism for controlling the level of deepest penetration of glandular elements into adjacent structures.

The significance of the loss of a control mechanism for limiting gland penetration would be to endow the glands with the potentiality of continued growth into surrounding tissues. One distinction between benign and malignant gland forms would thus be defined in terms of an inability of the benign forms to penetrate owing to the presence of a system fixing the relationship of the deepest glandular components to that of the surrounding normal tissues. The existence of this

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\(^2\) Unpublished experiments by L. W. Wattenberg.
fixed rim thus would result in an expansive type of growth occurring in an upward and lateral direction. On the other hand, in malignant forms orientation is lost, and an infiltrative type of growth occurs.

In malignant polyps, epithelium with weak succinic dehydrogenase activity is found in basal gland areas, and in more advanced malignant lesions low activity is frequently evident in the elements of the infiltrating margin of the tumor. In both instances proliferating epithelium, no longer restricted by the control mechanisms postulated to occur in basal cells of nonmalignant lesions, is in immediate relationship to underlying normal structures and thus has the potentiality of unlimited penetration into these structures. However, even with this potentiality, certain additional alterations in the metabolic pattern of the cells would almost certainly be necessary in order for them to survive and proliferate under conditions extant for infiltrating carcinoma. The increase in both DPN diaphorase and TPN diaphorase activities which have been found to occur in components of manifestly infiltrating carcinoma of the large bowel (15) would appear to reflect an aspect of such a metabolic pattern of malignancy.

In malignant polyps containing in situ carcinoma, epithelial elements have been observed deep within the polyp which do not appear malignant by classical morphologic criteria and yet show either a uniformly low succinic dehydrogenase activity or intimately related zones of high and low succinic dehydrogenase activity. This epithelium can be traced into adjacent areas which morphologically show unquestionable malignancy and where a low succinic dehydrogenase is also present. It would appear likely that the small focal decreases in succinic dehydrogenase activity in this nonmalignant-appearing epithelium are an incipient evidence of carcinogenesis. When such glandular components do not show other evidence of malignancy, as for example an increase in diaphorase activity, they probably represent transition forms which have undergone one, but not all, of the metabolic changes essential to malignant behavior, a phenomenon which could be termed "partial malignant transformation." Virtually all gradations between this state and morphologically manifestly malignant carcinoma in situ exhibiting low succinic dehydrogenase activity and high DPN diaphorase and TPN diaphorase activities can be seen in malignant polyps of the large intestine, presumably reflecting different metabolic stages in the conversion of cells to a completely malignant state.

SUMMARY

A morphologic histochemical study of succinic dehydrogenase and cytochrome oxidase in normal mucosa and proliferative lesions of the large bowel mucosa including focal hyperplasia, benign adenomatous polyps, malignant adenomatous polyps, and infiltrating carcinoma has been carried out. A decrease in staining for succinic dehydrogenase and cytochrome oxidase occurred in elements of all the proliferative lesions studied, and this appears to reflect an aspect of a metabolic pattern common to both benign and malignant proliferative processes of the large bowel mucosa.

In benign and in well differentiated malignant lesions, there were elements with high as well as elements with low succinic dehydrogenase activity. In the benign lesions, a clearly defined orientation exists in which weak activity occurred super-
Fig. 8.—Infiltrating carcinoma of the large intestine. Succinic dehydrogenase. Both light and relatively dark staining tumor components are present. Note the irregular reactivity present in some of the gland elements with light and darker staining zones of varying sizes interspersed. ×120.

Fig. 9.—Malignant adenomatous polyp. Succinic dehydrogenase. The field depicted is at the base of the polyp. The right half includes elements of atypical glands with irregular staining whereas, in the left half, more uniformly deep staining is present. ×80.

Fig. 10.—Malignant adenomatous polyp. Same block as Figure 9. DPN diaphorase. The gland elements corresponding to these showing irregular succinic dehydrogenase activity, Figure 9, have a high DPN diaphorase activity, those with more uniformly intense succinic dehydrogenase activity have a weak DPN diaphorase activity ×80.
Fig. 11.—Malignant adenomatous polyp. Hematoxylin and eosin. In the lower central portion of the field the epithelium is manifestly malignant as evidenced by its gross disorganization. X40.

Fig. 12.—Malignant adenomatous polyp. Same block as Figure 11. Succinic dehydrogenase. Much of the morphologically malignant appearing epithelium in the lower central portion of the field stains lightly, irregular staining of surrounding glandular elements is present. X40.

Fig. 13.—Malignant adenomatous polyp. Higher power view of lower central portion of the field of Figure 11. Hematoxylin and eosin. Note the transitions between the well organized epithelium which morphologically does not appear malignant and the disorganized manifestly malignant glands occupying most of the right upper portion of the field. X140.

Fig. 14.—Malignant adenomatous polyp. Higher power view of lower central portion of Figure 12. Succinic dehydrogenase. Note that the glands in the left half of the field, while morphologically appearing nonmalignant, Figure 13, are manifesting evidence of carcinogenesis as indicated by the irregular loss of succinic dehydrogenase activity. X140.
ficial to more intense activity. A loss of this orientation occurred in malignancy and may provide early evidence of carcinogenesis in glandular components which do not appear malignant as judged by conventional morphologic features.

In the cells at the base of the glandular crypts of the normal mucosa, hyperplastic mucosa, and benign adenomatous polyps, a high succinic dehydrogenase activity was observed, but the cytochrome oxidase activity was not correspondingly elevated. This observation suggests that the relationship between succinate oxidation and enzymes involved in electron transport differs in these sites from that of the remainder of the cells of these tissues.

It is suggested that succinate oxidation associated with a relatively weak cytochrome oxidase activity may reflect a mechanism for control of gland penetration into surrounding structures; and that, in carcinogenesis of the mucosa of the large bowel, a loss of this control mechanism occurs.

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