Enzyme Histochemical Changes in a Canine Thyroid Carcinoma Cell Line Inoculated with a Canine Lymphoma Suspension

E. H. FOWLER, L. KASZA, AND A. KOESTNER

Department of Veterinary Pathology, The Ohio State University, Columbus, Ohio

Summary

The canine thyroid carcinoma cell established in tissue culture was inoculated with a lysed-cell suspension of canine malignant lymphoma. In the current investigation, the activities of 11 selected enzymes of the oxidative and hydrolytic group were studied histochemically. Except for minor variations, the activity of each of the enzymes in the uninoculated cells remained constant throughout the growth period and into the early degenerative stages. The activity of the glycolytic and Krebs cycle enzyme systems was stronger than the activity of the succinocidase and electron transport system enzymes. Alkaline phosphatase activity varied less in the older cultures than in the younger ones.

It was concluded that, except for minor variations, the enzyme activity of the canine thyroid carcinoma cell was similar to the activity of other established tumor cell lines in tissue culture.

In the lymphoma-inoculated cultures, enzymatic alterations paralleling morphologic changes were demonstrated. These changes consisted of decreased activity of glucose-6-phosphate, glutamate, and lactate dehydrogenases, cytochrome oxidase, and alkaline phosphatase in the younger cultures, namely 48, 96, 144, and 168 hr. In the older inoculated cultures, 240, 288, and 336 hr, where the morphologic differences were no longer apparent, the only enzymes that continued to show less activity were glucose-6-phosphate dehydrogenase and alkaline phosphatase.

The possibility is discussed that these morphologic and enzymatic changes in the lymphoma-inoculated cells are due to an oncogenic virus effect in the early phase of growth.

Introduction

The growth and chromosomal pattern of a canine thyroid carcinoma cell line has been described (16, 28). In an attempt to isolate an oncogenic virus, a lysed-cell suspension of tonsil from a dog with malignant lymphoma was inoculated into these cells during their 38th passage. Morphologic alterations were demonstrated initially in the 17th postinoculation passage and maintained in all subsequent passages and chromosomal aberrations were detected.

The objectives of this investigation were to establish histochemically the enzymatic profile of the canine thyroid carcinoma cell in vitro and determine the changes induced by inoculation with a lysed-cell suspension of lymphoma material.

Materials and Methods

The methods of cultivation of canine thyroid adenocarcinoma cells have previously been described (16). An enlarged tonsil, surgically excised from a 6-year-old female Boxer dog with generalized malignant lymphoma, was homogenized, the cells were lysed, and a 20% suspension of this homogenate was inoculated into the canine thyroid carcinoma cell line.

This enzyme histochemical study was initiated on the 200th passage of the uninoculated and 162nd passage of the lymphoma-inoculated canine thyroid carcinoma cell culture. The studies were conducted at different stages during the growth cycle of the cells, namely 48, 96, 144, 168, 240, 288, and 366 hr. All enzyme histochemical procedures were carried out in Leighton tubes in which the cultures were grown. The cells were gently washed twice with physiologic saline prior to incubation in the substrates [Fortelius et al. (10)].

The activity of the following selected oxidative and hydrolytic enzyme systems was studied histochemically: glucose-6-phosphate dehydrogenase; glutamate dehydrogenase; lactate dehydrogenase; malate dehydrogenase; NADH cytochrome c reductase; NADPH cytochrome c reductase; succinate dehydrogenase; cytochrome oxidase; adenosine triphosphatase; alkaline phosphatase; and acid phosphatase.

The activities of G6PDH, GDH, LDH, and MDH were determined by a method modified from Hess et al. (15), utilizing nitro BT as the tetrazolium salt. The respiratory inhibitor, either azide or cyanide, was omitted since the tetrazolium salt employed was capable of capturing the electrons without additional blockage of the respiratory pathway (Scarpelli, personal communication, 1964). The activity of SDH was determined by the method of Nachlas et al. (23). The activities of NADH- and NADPH cyt. c red. were determined by a method modified from Scarpelli et al. (32). The osmolarity of the solutions was maintained at 0.44 M by the addition of sucrose to the incubating media.

Cytochrome oxidase activity was determined by the method of Burstone (5). Control sections were placed in a substrate

1 The following abbreviations are used: NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; nitro BT, nitro blue tetrazolium; NADH cyt. c red., NADPH cytochrome c reductase; NADPH cyt. c red., NADPH cytochrome c reductase; SDH, succinate dehydrogenase; CYO, cytochrome oxidase; ATPase, adenosine triphosphatase; ALP, alkaline phosphatase; ACP, acid phosphatase; and PAS, periodic acid-Schiff.

Received February 28, 1966; accepted June 17, 1966.

DECEMBER 1966
E. H. Fowler, L. Kasza, and Koestner

mixture containing potassium cyanide which blocked the respiratory pathway.

The activity of ATPase was demonstrated by the method of Wachstein et al. (34). Both ALP and ACP were demonstrated by simultaneous coupling azo dye techniques according to the method of Grogg and Pearse (12). Sodium α-naphthyl phosphate was used as the substrate for both reactions; Fast Violet B salt was used as the coupling agent for ALP; while Fast Garnet GBC salt was used for ACP.

Acetone (60%) for 30 sec at room temperature was used as the fixative for the dehydrogenase and diaphorase procedures (29). The activity of cytochrome oxidase was determined on unfixed cells. For ATPase, the fixation consisted of 10% formaldehyde and rimmed with clear nail lacquer. Following fixation, the cells were rinsed with saline at -2°C for 5 min, and for ALP and ACP, the cells were fixed in 4% neutral formalin at 4°C for 15 min and 60 min, respectively (10). Following fixation, the cells were rinsed with physiologic saline prior to incubation in the substrate mixture.

All incubations were conducted at 27°C, except ALP and ACP, which were carried out at room temperature. ALP reactions were incubated 45 min, G6PDH, LDH, NADH cyt. c red., and CYO reactions were incubated 90 min; GDH, MDH, ACP, and ATPase reactions were incubated 120 min; and SDH and NADPH cyt. c red., reactions were incubated 180 min.

Following incubation, the cover slips were rinsed in distilled water, removed from the Leighton tubes, mounted with glycerol-jelly and rimmed with clear nail lacquer.

Sections of rat liver or kidney were incubated in each substrate mixture in order to control for false-negative reactions. All of the reactions except cytochrome oxidase were controlled by incubating the cells without the substrate.

Additional histochemical procedures consisted of the May-Grunwald-Giemsa stain for general cellular morphology, the toluidine blue reaction for metachromasia, the PAS reaction for free aldehyde groups, the Feulgen reaction for DNA, and the methyl green-pyronine stain for RNA and DNA. These sections were mounted in Piccolyte, a synthetic resin (General Biologicals, Chicago) prepared in a 60% solution with xylene.

Results

The uninoculated canine thyroid carcinoma cells were epithelioid in appearance and varied in size and shape during the early stages of their growth cycle. Giant cells, both mono- and multinuclear, were observed and mitoses were frequently encountered (Fig. 1). The cytoplasm was slightly granular and possessed few vacuoles. The cytoplasmic periphery was basophilic while the juxtanuclear area was azurophilic. The cytoplasm frequently possessed small azurophilic granules, when stained with May-Grunwald-Giemsa, which were PAS-positive and diastase-sensitive. Cytoplasmic RNA was revealed with the methyl green-pyronine stain, but little nuclear DNA was observed with the Feulgen reaction.

Between 144 and 168 hr, the monolayer became confluent, the cells had reached maturity, were smaller and more uniform in size and shape, and mitoses were less apparent. A few individual degenerating cells were observed. The cytoplasm was more vacuolated, and the azurophilic area next to the nucleus was less prominent (Fig. 2). The small, azurophilic, PAS-positive granules were still present in the cytoplasm. Between 240 and 288 hr, more degenerating cells were observed, the monolayer became less uniform and giant cells were again observed. Cells of various shapes and sizes were seen in actively proliferating areas of these cultures (Fig. 3). In the 240- to 336-hr cultures, the cytoplasm was completely vacuolated and lacked the azurophilia observed in the younger cultures. Small azurophilic granules were occasionally present.

Morphologic alterations in the lymphoma-inoculated cells were observed as early as 48 hr of growth. The cells were smaller, more uniform in size, and possessed more cytoplasmic processes than the uninoculated cells. No juxtanuclear azurophilia was present, and cytoplasmic vacuolation appeared earlier. Fewer giant cells were observed, the cells tended to pile up, and they possessed a decreased ability to adhere to the surface of the cover slip. At 168 hr, more degenerating cells were observed than in the uninoculated controls. The cells in the middle of the clumps were smaller than those at the periphery.

In the cultures 240 hr and older, the morphologic differences between the lymphoma-inoculated and uninoculated cells were no longer apparent.

The enzyme activity of the uninoculated and lymphoma-inoculated canine thyroid carcinoma cells at different stages of development is illustrated in Table 1. The degree of enzyme activity was subjectively graded from 0 to +++++.3

In all of the enzyme preparations, the activity varied among the individual cells. This was particularly apparent in the LDH, MDH, and NADH cyt. c red. reactions, or with enzymes which possessed intense activity (Fig. 4). In the procedures using the tetrazolium salt, nitro BT, large, dark granules were observed in the cytoplasm. These granules varied in size and number and were distinct from the smaller, lighter-colored granules in the rest of the cytoplasm. These dark granules were eliminated when 100% acetone was used as the fixative, but the enzyme activity was then decreased. In nearly all enzyme procedures performed on the young cultures, more activity was observed on the edge of the cover slip than in the center.

Glucose-6-phosphate Dehydrogenase

In the uninoculated control cultures, G6PDH activity was generally strong and concentrated on 1 side of the nucleus. There was more variation between the cells in the younger cultures, and the number of dark granules increased with age. The giant cells possessed strong activity, and scattered small cells demonstrated intense activity. In the lymphoma-inoculated cultures, these was less G6PDH activity in most age groups, with some increase in activity in the older cultures (Figs. 5, 6).

Glutamate Dehydrogenase

In the uninoculated cultures, there was less lighter blue color in the cytoplasm than observed in the G6PDH reactions. Phase contrast revealed many G6D inactive cells in the 96-144-hr cultures. This enzyme was moderately active in all age groups and intense in the smaller cells. In the lymphoma-inoculated cultures, for the 1st 168 hr, only weak GDH activity was demonstrated.

3 The grading of enzyme activity was based on the following scheme: 0, no activity; +, weak activity; ++, moderate activity; ++++, strong activity; +++++, intense activity.
Histochemical Changes in Inoculated Carcinoma Cell Line

TABLE 1
Enzyme Histochemical Activity of 3 Representative Stages of Growth of the Uninoculated and Lymphoma-inoculated Canine Thyroid Carcinoma Cells

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>48 HR</th>
<th>144 HR</th>
<th>288 HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninoculated</td>
<td>Lymphoma inoculated</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>G6PDH*</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>GDH</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>LDH</td>
<td>++++</td>
<td>0-+++</td>
<td>++++</td>
</tr>
<tr>
<td>MDH</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>NADH cyt. c red.</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>NADPH cyt. c red.</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>SDH</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>CYO</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>ATPase</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>ALP</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>ACP</td>
<td>+</td>
<td>0-+++</td>
<td>0-+++</td>
</tr>
</tbody>
</table>

* Abbreviations: G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; NADH, cyt. c red., reduced nicotinamide adenine dinucleotide cytochrome c reductase; NADPH cyt. c red., reduced nicotinamide adenine dinucleotide phosphate cytochrome c reductase; SDH, succinate dehydrogenase; CYO, cytochrome oxidase; ATPase, adenosine triphosphatase; ALP, alkaline phosphatase; ACP, acid phosphatase; 0, no activity; +, weak activity; ++, moderate activity; +++, strong activity; ++++, intense activity.

After 240 hr, when the cells became morphologically similar to those of the uninoculated cultures, the GDH activity was also the same.

Lactate Dehydrogenase

Lactate dehydrogenase activity in the uninoculated cultures was strong in all phases of growth (Fig. 7). Mononuclear giant cells and scattered small cells possessed intense activity in the 48-hr cultures. In the lymphoma-inoculated cultures, the LDH activity was low in the 48- to 144-hr cultures (Fig. 8) and increased to the level of the controls at about 240 hr of age.

Malate Dehydrogenase

Malate dehydrogenase activity was higher than LDH activity, both in the uninoculated and lymphoma-inoculated cells. There was more variation between the individual cells in the 96- to 144-hr cultures. Some cells demonstrated little to no activity while other cells possessed intense activity (Fig. 4). The activity of MDH in the lymphoma-inoculated cells was nearly the same as in the uninoculated controls.

NADH- and NADPH Cytochrome c Reductase

The activities of NADH- and NADPH cyt. c red. were nearly the same in the uninoculated and lymphoma-inoculated cultures, NADH cyt. c red. being slightly stronger. At 48 hr, all cells showed strong to intense activity, the reaction being concentrated on 1 side of the nucleus (Fig. 9). Between 96 and 144 hr, only 50% of the cells possessed enzyme activity, the rest being demonstrable only by phase contrast. In the 240- to 336-hr cultures, the enzyme activity again became more uniform.

Succinate Dehydrogenase

SDH activity was similar in the uninoculated and inoculated cultures. The enzyme activity was moderate to strong in all age groups (Fig. 10); however, older cultures (240–336 hr) possessed many cells with weak activity. Giant cells had less activity than the smaller cells.

Cytochrome Oxidase

Cytochrome oxidase activity was concentrated on 1 pole of the nucleus, similar to the activities of G6PDH, NADH-, and NADPH cyt. c red. The activity was fairly uniform in most uninoculated cultures and ranged between moderate and strong (Fig. 11). Intense activity was often observed in cells located in the regions of active cell proliferation. In the 240–336-hr cultures, many cells with weak activity were observed. CYO activity was weak in the young inoculated cultures (Fig. 12) and increased with age to the levels of control cultures.

Adenosine Triphosphatase

The activity of ATPase was primarily concentrated in the cell membranes (Fig. 13); however, some mitochondrial activity was present. Weak to moderate activity was observed in the young cultures with a slight increase as the monolayer became confluent. The activity of ATPase in the lymphoma-inoculated cultures was slightly greater than in the uninoculated controls.

Alkaline Phosphatase

Alkaline phosphatase activity was variable, particularly in the young uninoculated cultures. Some cells possessed little to no activity while others demonstrated moderate to strong activity (Fig. 14). There was an increase in the number of active cells as well as of the degree of activity in the older cultures (Fig. 15). No ALP activity was demonstrable in the young lymphoma-inoculated cultures. It increased in focal areas as the morphologic variations in the 2 cultures disappeared (Fig. 16) but remained always lower than in the uninoculated cells.

DECEMBER 1966
Acid Phosphatase

Acid phosphatase activity was uniformly weak to moderate in the young uninoculated cultures with increased activity in individual cells in older cultures. ACP activity was slightly stronger in the lymphoma-inoculated cells.

Discussion

The uninoculated canine thyroid carcinoma cells in tissue culture appeared similar to cultivated normal cells after the cell line became established, in that the cells produced a uniform monolayer at a constant rate when grown in bottles or Leighton tubes. The enzyme activity in the uninoculated cells showed some variation between the cells in many preparations, but little significant variation was present in cultures of different ages.

The oxidative enzymes functional in the succinoxidase and electron transport systems, namely SDH, CYO, NADH-, and NADPH cyt. c red. were less active than the enzymes functional in glycolysis and the Krebs cycle, namely G6PDH, LDH, and MDH. High LDH activity and relatively low SDH activity were found in 3 permanent cell lines by Leslie and Yarnell (19). They also reported that SDH was entirely located within the mitochondria, which would account for the granular appearance of SDH activity in the current investigation.

Leukemia viruses have been demonstrated to propagate in tissue-cultured cells without producing gross cytopathic changes (11, 22). Morphologic changes were induced in the lymphoma-inoculated thyroid carcinoma cells which consisted of alterations in cell size, piling up of cells in clumps and a decreased adhesiveness of the cells to the cover slip. These changes are consistent with the growth of malignant cells in tissue culture (2). These morphologic changes were paralleled by enzymatic alterations, specifically a decrease in the activity of G6PDH (hexose monophosphate cycle), LDH (glycolytic pathway), GDH (amino acid synthesis), CYO (cytochrome system), and ALP (a hydrolytic enzyme). Little to no effect was observed on MDH (Krebs cycle), SDH (succinoxidase system), NADH-, and NADPH cyt. c red. (electron transport), ATPase (phosphorylation), or ACP (lysosomal enzyme). Raikhlin and Staroverova (30) reported a decrease in SDH and CYO activity in fibroblasts undergoing malignant transformation while in the current investigation these enzymes were relatively unaffected. LDH activity is usually increased in neoplastic cells rather than decreased as observed in this study.

In cells infected with some cytopathic viruses, Kaufman and Hill (17) reported a decrease in SDH activity prior to morphologic changes whereas Fortelius (9) found no changes in oxidative enzyme activity before distinct morphologic cell degeneration was apparent; however, no glycolytic enzymes were studied.

The decreased G6PDH activity in the lymphoma-inoculated cells was present in all age groups, even after the morphologic differences were no longer apparent. This decreased G6PDH activity suggested decreased RNA production in the inoculated cells. Glycolytic activity was altered by decreased LDH activity in the younger inoculated cells, but was not found to be different in the older cultures.

The moderate to strong SDH and CYO activity present in the uninoculated cells is similar to that found in most continuous cell cultures (8, 9, 30). SDH activity was apparently not affected in the lymphoma-inoculated culture, whereas the CYO activity was decreased in the young inoculated cells.

The decreased activity of CYO, together with the decreased activity of LDH, suggests a decreased metabolic rate compared with the control cells, which would account for the decreased prominence of the Golgi apparatus.

The absence of ALP activity in the young lymphoma-inoculated cultures explains the lack of adhesiveness of the cells to the glass surfaces since ALP activity in the membranes of cells is necessary for proper adhesion (13). Decreased ALP activity has been demonstrated in cells infected with leukemia virus (33), in cells with a loss of chromosomes (16), and in cells infected with cytopathic viruses (3, 4). Fortelius, on the other hand, did not observe any change in ALP activity in virus-infected cells (9).

The variation in ALP activity, found in the young uninoculated cultures in this study, has also been reported by Fortelius (8-10) and Keefe et al. (18) for other established cultures. The activity of ALP is dependent upon the length of time the cells have been cultured (13, 24) and may vary with alterations in the medium (3, 7, 14) or chromosomes (6). According to Ogawa et al. (27), ALP and ACP activity is present in the lysosomes, which gives a granular appearance to the cytoplasm. The ALP activity was less granular in the present investigation than was the ACP activity, and became more uniform and stronger in the older cultures. Fortelius reported a decrease in ALP activity in older cultures (9).

The slight increase of ACP activity in the lymphoma-inoculated cells was due to the increased number of scattered degenerating cells in the inoculated culture. It has been demonstrated that ACP activity does not vary during growth (7) and a slight decrease is noted in older cells (9) provided toxic effects are not present in the medium. Activation of lysosomal enzymes, specifically ACP, occurs in cells infected with some cytopathic viruses before (1) and after (4, 21) cytopathic effects are observed, whereas this activation is not present in cells infected with non-cytopathic viruses (21).

The morphologic similarity between the uninoculated and lymphoma-inoculated thyroid carcinoma cells in the older age groups suggests that no cytopathic effect was produced by the inoculum. The slight increase in the number of scattered degenerating cells in the older lymphoma-inoculated cultures was not considered significant. No significant increase in ACP activity was present. The oxidative enzyme activity in the older inoculated cultures suggested similar metabolic rates in the inoculated and uninoculated cells.

The PAS-positive azurophilic material and granules present in the cytoplasm of the young uninoculated cultures was an indication of the active metabolism in these proliferating cells. This feature was lacking in the lymphoma-inoculated cells. The increase in cytoplasmic vacuolation in the older cells was due to the lipid which Kasza (16) demonstrated in the cytoplasm of the thyroid carcinoma cells. This lipid accounts for the presence of the dark bluish-black granules in the cytoplasm of the cells stained for the demonstration of dehydrogenases and diaphorases. Novikoff et al. (26) have shown that reduced tetrazolium salts will precipitate on lipid interfaces and that this lipid must be removed for proper localization of enzyme activity. The 60% acetone fixation, while removing some of the lipid, did not suc-
Histochemical Changes in Inoculated Carcinoma Cell Line

Therefore, the 60', o acetone, described by Quagliano and Hayhoe (29) and recommended by Fortelius (9, 10), was employed as the fixative of choice.

The increased activity observed at the periphery of the cover slip in many of the reactions was due to the increased metabolic activity and growth potential of the cells in this area. As the monolayer became confluent, the activity became correspondingly more uniform.

Concentration of enzyme activity on 1 side of the nucleus, described by Sandritter and Schiemer (31) and Fortelius et al. (8–10) was considered to be in the area of the Golgi zone. Novikoff and Goldfischer (25) found acid phosphatase activity in small granules in the region of the Golgi apparatus. The halo appearance of the enzyme activity observed in the uninoculated thyroid carcinoma cells corresponds to the azurophile halo and was evidence of an active Golgi apparatus. The lymphoma-inoculated cells apparently did not have an active Golgi apparatus since both of the above-mentioned features were absent.

In all of the dehydrogenase and diaphorase reactions, there were small cells which possessed more activity than the surrounding larger cells. Fortelius (8) explains this on the basis of condensation of the cytoplasm which results in concentration of enzyme activity.

Fortelius studied the enzyme histochemical activity of ALP, ACP, EST (nonspecific esterase), CYO, SDH, and AP (aminopeptidase) in 7 continuous cell lines and 2 primary cultures (9, 10). He stated that the enzyme activities did not show much variation in the different cell lines. Lieberman and Ove (20) also found a similarity in the activity of ACP, β-glucuronidase, catalase, NADH cyt. c red., esterase, G6P, G6PDH, LDH, and xanthine oxidase in 4 human cell cultures.

The variations in enzyme activity observed among the cells are supported by 2 studies conducted 1 year apart by Fortelius (8, 9) who considered such variations to be legitimate evaluations. The greatest difference was observed in the 96- and 144-hr cultures and it seemed to disappear in the older cultures. This feature was not as prominent in the cultures fixed with 100% acetone. It was surmised that this variation in enzyme activity might be due to the resistance of the cell membranes to fixation which consequently affected the permeability of the membranes to the substrates. This membrane resistance decreased when the culture matured and the monolayer became confluent. The uniform activity in the cells fixed in 100% acetone suggests that the fixation was sufficient to make the membranes freely permeable to the substrate; however, the amount of enzyme activity was decreased.

It can be concluded that the uninoculated canine thyroid carcinoma cell possesses enzyme activities similar to other established tumor cell lines. The presence of variable activity in the young, rapidly proliferating cells might suggest that the confluent monolayer composed of mature cells would be the best stage at which to examine enzyme changes.

The morphologic and enzyme histochemical changes present in the lymphoma-inoculated cells suggests a decrease in metabolic activity, especially associated with nucleic acid formation, anaerobic glycolysis, and electron transport. These alterations, which are present in the early stages of growth in each successive generation, usually disappear as the culture matures which suggests a transient rather than a permanent or genetic inhibition. Although there is no evidence to support this hypothesis, this suggests that a noncytopathic agent, presumably a virus, is present in these cells causing an alteration in the metabolic activity during the initial phases of cell multiplication, which would also be during the stage of agent replication. When the culture matures, and the agent has reached its optimum production to live in symbiosis with the cell, the metabolic activity of the cell increases to the level observed in the controls.

References


17. Kaufman, N., and Hill, R. W. Sucinie Dehydrogenase Activ-
Carinoma cells. X 405.

E. H. Fowler, L. Kasza, and Koestner

Carcinoma cells. May-Grünwald-Giemsa stain, X 405.

FIG. 4. Marked variability of MDH activity in a 96-hr uninoculated culture of canine thyroid carcinoma cells. Phase contrast, X 405.

FIG. 5. Intense G6PDH activity in a 336-hr uninoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 6. Moderate G6PDH activity and numerous dark bluish-black granules in a 336-hr lymphoma-inoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 7. Intense LDH activity and many dark granules in a 48-hr uninoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 8. Weak to moderate LDH activity in a 48-hr lymphoma-inoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 9. Juxtanuclear localization of NADH cyt. c red. activity in a 144-hr uninoculated culture of canine thyroid carcinoma cells. X 640.

FIG. 10. Granular appearance of SDH activity in a 144-hr uninoculated culture of canine thyroid carcinoma cells. X 640.

FIG. 11. Moderate to strong CYO activity in a 96-hr uninoculated culture of canine thyroid carcinoma cells. X 640.

FIG. 12. Weak CYO activity in a 96-hr lymphoma-inoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 13. Strong ATPase activity in the cellular membranes of a 288-hr uninoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 14. Variable ALP activity in a 168-hr uninoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 15. Strong slightly variable ALP activity in a 288-hr uninoculated culture of canine thyroid carcinoma cells. X 405.

FIG. 16. Moderate to strong focal ALP activity in a 288-hr lymphoma-inoculated culture of canine thyroid carcinoma cells. X 405.
Histochemical Changes in Inoculated Carcinoma Cell Line
Enzyme Histochemical Changes in a Canine Thyroid Carcinoma Cell Line Inoculated with a Canine Lymphoma Suspension

E. H. Fowler, L. Kasza and A. Koestner

Cancer Res 1966;26:2409-2418.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/26/12_Part_1/2409

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/26/12_Part_1/2409. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.