Energy Mechanisms in Malignant Tumors in Relation to Chemotherapy*

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Differences in metabolism of neoplastic tissue and its normal homologue have been the subject of extensive experimental work. Burk (4) has suggested seven criteria of malignant metabolisms, while the studies of Greenstein (8) have done much to map the enzymatic patterns of malignant tumors. These studies as well as many others have pointed to the accentuation of both aerobic and anaerobic glycolysis as well as to a diminution in the constituents and reactivity of the respiratory chain of enzymes viz., flavoprotein, cytochrome and cytochrome oxidase. Crystallization of these observations is found in the statement by Greenstein (8, p. 201) that “tumors tend to converge enzymatically to a common type of tissue.”

The differences in metabolism between normal and malignant tissues have been the basis for many attempts to develop a chemotherapeutic approach to their control. Direct observation in tissue culture of differential sensitivity to various enzyme inhibitors was reported by Chambers, Cameron and Kopac (5).

Attempts to inhibit the glycolytic activity of tumor tissue in vitro have in some instances been encouraging (13, 3, 15). When given to the intact animal, however, these enzyme inhibitors in general have been found ineffective (12, 17, 11) except in a few instances (6, 7, 19).

The possibility of a new approach toward a rational chemotherapy of malignant tumors seemed to be indicated in terms of the energy-rich phosphate bond. The importance of these bonds in relation to cellular energy requirements has been stressed by Lipmann (14) and Kalckar (10). If malignant cells are particularly dependent on the glycolytic mechanism for their energy requirements it might be possible to inhibit their activity if one could selectively limit the formation or utilization of these bonds.

Based on this concept, an attempt was made to create a tentative model of biochemical function in malignant tissue as compared to the normal. It was felt that with the advent of malignant neoplasia there was a significant alteration in the energy mechanism of tissue. The preferred pathways would now involve the glycolytic mechanism while the respiratory enzyme reactions would be diminished or be of limited importance as compared to their role in normal tissue. It should be stressed that the respiratory functional potentiality is not absent but merely residual. This is of particular importance from the point of view of mechanisms of adaptation. The glycolytic mechanism itself may be divided into two main groups, the primary portion from triose to lactic acid and the tricarboxylic acid cycle which would serve as an accessory or secondary mechanism for energy production.

The value of any model or theory rests to a large extent on its ability to indicate significant experiments or to prognosticate and unify observations. On the basis of this hypothesis the following would be predicted.

1. The preferred sites for inhibiting the primary glycolytic mechanism in order to obtain maximum destruction of energy-yielding reactions in malignant tissue. These would occur at the points of creation of the high energy phosphate bonds, namely the coupled oxidation-phosphorylation of 3-phosphoglyceraldehyde to 1,3-diphosphoglyceric acid, and the enolization of 2 phospho pyruvic acid to phospho-enol pyruvic acid. These reactions are inhibited respectively by iodoacetic acid and sodium fluoride.

2. That such inhibition would have minimal effects on normal tissue.

3. That adaptation of malignant tissue to these inhibitors would occur. This would be associated with utilization of a secondary mechanism for energy production.

(a) The tricarboxylic acid cycle is a likely secondary pathway. Inhibition of the cycle could be accomplished by the use of malonic acid which inhibits the succinic dehydrogenase system.

(b) Further adaptation after initial sensitivity of the malignant tissue to the glycolytic inhibitors would occur. This might well be accomplished...
was determined by studies of the therapeutic effects of the inhibitors and the therapeutic dose of one of them it is possible to compute the therapeutic doses of the others.

The acute lethal doses were obtained from the data of Handler (9) who used intraperitoneal injections in rabbits. He obtained the following values for the various inhibitors: sodium fluoride 250 mgm./kgm., sodium iodoacetate 80 mgm./kgm., sodium malonate 1,500 mgm./kgm., sodium azide 10 mgm./kgm. For our calculations the value of 320 mgm./day was chosen for the average therapeutic dose of sodium fluoride for an adult. This was determined by studies of the therapeutic effects on leukemia cases to be described below. From these figures simple ratios were set up in the following form.

\[
\text{Lethal dose X \over \text{Lethal dose Y}} = \text{Therapeutic dose X \over \text{Therapeutic dose Y}}
\]

The values for iodoacetic acid and malonic acid were determined in this manner. In the case of sodium azide the value arrived at was divided by 3, in view of observations that the cytochrome oxidase content of tumors is roughly in a 1:3 ratio with normal tissue (8, p. 249). The comparisons of the calculated doses with the doses found to be clinically effective are listed in Table I.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Calculated dose* mgm.</th>
<th>Clinically effective dose* mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoride</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>60-90</td>
<td></td>
</tr>
<tr>
<td>Malonic acid</td>
<td>1000-1500</td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>2.7-2.5</td>
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* Dose/day for average adult.

This report gives the results of preliminary experiments based on the concepts mentioned above. The various inhibitors were tested on cases of acute leukemia as well as various types of malignant tumors. Acute leukemia was chosen initially as a particularly good subject for study in view of the uniformly poor prognosis and the ease of following therapeutic effects by means of peripheral blood counts. While there are reports of occasional spontaneous remissions in acute leukemia, these are rare. Further, the remissions are not predictable as to time and method of occurrence. The essential validity of the foregoing predictions is indicated by the beneficial therapeutic effects observed in a significant number of the patients treated.

The various drugs were given orally in divided doses. In the case of sodium fluoride it was necessary to prevent the formation of hydrofluoric acid in the stomach. This was achieved either by the use of enteric coated tablets or by the simultaneous administration of amphoteric antacids.

**Acute myeloblastic leukemia.**—Ten cases were studied, 5 of which showed definite clinical and hematological improvement, 2 showed some improvement coincident with therapy, while 3 experienced little or no effect. Improvement, when it occurred, consisted of gain in strength, appetite and alertness, and decrease in blasts, adenopathy and hepatosplenomegaly.

Of particular interest was the case of L. G., a 3½ year old white girl. Administration of sodium fluoride and iodoacetic acid (together) was accompanied by disappearance of blasts from the peripheral blood. Adenopathy and hepatosplenomegaly disappeared within a month. Following cessation of therapy for 2 weeks, there was a recurrence of the original symptoms and adenopathy. Resumption of treatment with sodium fluoride and iodoacetic acid failed to bring any regression in the adenopathy or hepatosplenomegaly, or to improve the clinical status. It was assumed that the cells had become adapted to these inhibitors and were now using an accessory pathway of metabolism. The tricarboxylic acid cycle was considered to be the next mechanism most likely to be available. The use of malonic acid was decided upon because of its known ability to inhibit the succinic acid dehydrogenase system. Within a week of the institution of malonic acid, in conjunction with the other two inhibitors, the glands became soft and fluctuant, and were resorbed. After about a month, during which all three inhibitors were administered, there was a recurrence of hepatosplenomegaly, glands and blasts. The child died shortly thereafter from pulmonary hemorrhage, apparently due to a lack of platelets. The total length of observation was 5 months.

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It is particularly noteworthy that although the glycolytic inhibitors were effective initially, a process of adaptation appeared to take place so that the initial sensitivity was lost. It was felt that adaptation might have been accomplished by the use of reactions involving the "diminished metabolic pathway" as indicated in Fig. 1. Alteration in the leukemic cell was also suggested by the observation that morphological changes occurred in the leukemic cell after exposure to the glycolytic inhibitors coincident with the development of resistance to these drugs. The original myeloblastic cell usually possesses a well defined basophilic cytoplasm, and a vesicular nucleus which contains several distinct nucleoli. Leukemia patients having such cells were almost uniformly sensitive to the glycolytic inhibitors, sodium fluoride, iodoacetic acid and malonic acid. Treatment resulted in disappearance of adenopathy, hepatosplenomegaly and elimination of these malignant cells from the peripheral blood. However, when adaptation occurred, as indicated by recurrence of adenopathy and elevation of the white count, it was noted that a transition had occurred from the original blast cell to a cell which looked very much like an unusual lymphocyte. It varied in size from 8 to 20 μ in diameter and was characterized by an almost complete lack of cytoplasm. The nucleus possessed a condensed chromatin pattern in which nucleoli were either absent or reduced to small crescentic structures. These variations between the original cell type and the adaptation cell are illustrated in Fig. 2 and 3.

This relationship between metabolic sensitivity and morphological appearance of the leukemic cell is well illustrated by the following protocol.

S. R., (Fig. 4) 35 year old white male developed inguinal adenopathy in October, 1946. Biopsy was interpreted as lymphosarcoma; the peripheral blood count was normal. The patient received x-ray therapy to the groin and in addition was started on the glycolytic inhibitors. In spite of
continuous exposure to these drugs the patient seemed to be declining slowly. On December 24, 1946 a typical picture of acute myeloblastic leukemia was noted in the peripheral blood. This was corroborated by sternal-marrow aspiration. The malignant cells at this time were almost equally divided between two types, (a) a myeloblastic cell having well defined cytoplasm and a vesicular nucleus containing nucleoli, and (b) the adaptation type cell described above. In spite of continuous exposure to the glycolytic inhibitors (sodium fluoride 80 to 160 mgm. t.i.d., iodoacetic acid 30 mgm. t.i.d. and malonic acid 0.5 gm. t.i.d.) the patient showed slow but progressive development of adenopathy and hepatomegaly, the continued presence of malignant cells in the peripheral blood smear, absence of polys and general debilitation. At the same time it was noted that the malignant cells were becoming predominantly of the adaptation type. As can be seen from Fig. 3, there was little or no effect on the malignant cells, despite extensive and continued therapy with the glycolytic inhibitors. At the same time the clinical course was slowly down-hill, with progressive adenopathy. It seemed likely that in the face of the glycolytic inhibitors the malignant cells were using the diminished metabolic pathways which, as indicated in Fig. 1, involve oxidase activity. Therefore an attempt was made to evaluate the effect of an inhibitor of oxidase activity, namely, sodium azide.

The administration of the glycolytic inhibitors was discontinued on February 13 and sodium azide in doses of 0.625 mgm. t.i.d. started on February 17. This was continued for 10 days, during which time there seemed to be a slight decrease in adenopathy as well as some fall in the malignant cell count. The azide was then stopped and the glycolytic inhibitors re instituted in the following doses: sodium fluoride 80 mgm. q.i.d, iodoacetic acid 30 mgm. b.i.d. and malonic acid 0.5 gm. b.i.d. In the interim between the cessation of the azide therapy and the reintroduction of an effective concentration...
of the glycolytic inhibitors there was a marked increase in the malignant cell count and adenopathy. There was a coincident reappearance of nucleoli and basophilic cytoplasm in the blast cell. This was followed by an increase in polys and disappearance of malignant cells and adenopathy. Omission of therapy was again followed by reappearance of clinical discomfort and adenopathy and leukemia cells. These cells were of the original type, show-

Fig. 4

S.R. - Acute Myeloblastic Leukemia

Fig. 4.—The effect of the various inhibitors on case of acute myeloblastic leukemia.

mission, we found no correlation between any transfusion (more than 200 given) and clinical or hematological remission.

A rather uniform clinical pattern was observed in response to the therapeutic agents. Thus, clinical effect was not usually manifest until after three weeks of therapy. In the leukemia cases, however, some change in the blood count was usually noted as early as 24 hours after start of sodium fluoride therapy. This consisted of a decrease in the blasts either through drop in white count or appearance
of increased percentage of smudges with an associated drop in percentage of blasts. The exact mechanism was not predictable although it appeared that the blasts with abundant cytoplasm usually became smudges before the count dropped. This response may be so marked that the smudges may constitute as much as 85 per cent of the total count (Fig. 5).

Associated with the rapid destruction of the leukemic cells there is an elevation of temperature, which may go as high as 106° F. This usually lasts from 5 to 7 days; when it falls the adenopathy and hepatosplenomegaly are usually gone and the blood cleared of blasts. If the count previously consisted almost entirely of leukemic cells, there will be a marked leukopenia. Whether or not there will be return of the normal constituents depends on the bone marrow function. In general, the bone marrow response appears to be inversely proportional to the duration of the disease. So long as the blood remains free of malignant cells there is little evidence of the usual clinical features of agranulocytosis. In fact, the patient is usually clinically quite well.

The occurrence of hyperpyrexia associated with a therapeutic effect as seen in leukemias is usually not seen in other types of malignant neoplastic disease receiving this type of therapy.

Acute lymphatic leukemia.—Of 4 cases treated, all showed definite clinical and hematological improvement. One case treated with sodium fluoride alone underwent progressive changes, coincident with therapy, to what appeared to be a low grade chronic leukemia. She remained clinically well and active for 2 years. There was then a recurrence of the original acute type. Unfortunately circumstances prevented testing efficacy of the medication at this time. The patient succumbed.

Acute monoblastic leukemia.—In the single case studied, little change could be noted in the progressive decline over a period of 5 weeks' treatment with sodium fluoride, iodoacetic acid and malonic acid.

Gastric carcinoma.—Two cases were treated, both of which showed definite clinical improvement. In one, the large immobile epigastric mass initially present was converted within 5 weeks to a freely movable mass definitely smaller in size. The 70 year old patient died a cardiac death within 2 months. The other patient could not tolerate the medication after 4 weeks and therefore no effective doses were administered after that period. Death occurred from progression of the disease within 4 months.

Lymphosarcoma.—Coincident with treatment for 5 weeks, a retroperitoneal lymphosarcoma about the size of a man's head shrank to the size of a baseball. This dramatic lessening in mass was accompanied by clinical improvement. There followed a 2 week period of rest from therapy, during which the tumor again increased in size; subsequent resumption of therapy for 10 days was associated with a second decrease. An exploratory operation was now performed. The patient died of a retrograde peritonitis subsequent to the operative procedure. Surgical biopsy and postmortem microscopic examination revealed extensive and unusual necrosis and replacement fibrosis.

Carcinoma of the colon with metastasis to the liver.—Three cases showed a similar tendency for initial improvement in clinical status and some shrinkage of the enlarged livers within a month. However, these patients exhibited an inability to tolerate the medication beyond that period, experiencing nausea and vomiting. This was soon followed by a downward trend.

Carcinoma of the lung.—Four cases were studied
and all showed improvement coincident with therapy. Observations included decrease of pleural effusion, gain in weight and other signs of clinical improvement. One of these cases is still under observation, the others have died.

The following condensed protocol will serve to illustrate the findings in the former case of lung carcinoma.

W. K., 65 year old white male, having a long standing history of chronic bronchitis. Starting in November, 1946, the patient noted increasing weakness and distress. X-ray revealed atelectasis at the base of the right lung. Bronchoscopy disclosed a mass in the bronchus which proved to be squamous cell carcinoma. There had been a progressive weight loss of 17 pounds from November to December.

The patient was first seen by us on December 30, 1946, at which time he weighed 110% pounds. He was emaciated, had a chronic cough, poor appetite and gasping respiration. Hemoglobin was 9.0 gm. He was given all three glycolytic inhibitors, sodium fluoride, 80 mgm. t.i.d.; iodoacetic acid, 30 mgm. b.i.d.; and malonic acid. 0.5 gm. b.i.d. In addition he was started on a series of injections of testosterone propionate, 25 mgm. t.i.d.

Under this form of treatment there was a progressive gain in weight, strength and hemoglobin, so that by March 26, 1947 he weighed 124 pounds and had 14 gm. of hemoglobin.

Some loss of weight and reappearance of cough recurred which was checked by administration of sodium azide followed by the glycolytic inhibitors.

The patient died in June, 1947. Autopsy revealed hyperplasia of the poly series, with extensive purulent material in the atelectatic right lung had spilled over and occluded the left bronchus.

The use of testosterone in conjunction with the inhibitors in this case does not seem to have altered the general pattern of response to the inhibitors. The effects were neither accelerated or deterred. Further, the dose in this case was less than the dosage employed, had no apparent effect upon nor-mal cells. Thus in all cases of carcinoma treated with the same doses as the cases of leukemia there was no morphological change in any of the blood components. Similarly there was no apparent effect of inhibitor sodium fluoride on the clinical course or hematological picture in 4 cases of chronic myelogenous leukemia.

Carcinoma of the testis.—Two cases were treated.

The principal complaint of one patient was excruciating pain of sciatic type arising from metastasis in the right psoas area, proved by x-ray. Bed rest and opiates were of little avail. Within a month this patient was well enough to be ambulatory; he refused further treatment because it made him nauseous. (Both he and his wife were unaware of the diagnosis.) After a temporary period of well-being, his clinical status deteriorated rapidly. The second patient showed decrease in gynecomastia, relief from pain and increased strength. The 24 hour output of 17-ketosteroids decreased from 29.2 mgm. to 20.5 mgm. This patient had also an advanced case of diabetes, and died suddenly in what appeared to be diabetic coma. The total duration of study was 3 months.

Hodgkin’s disease.—Three cases were treated, all of whom showed definite clinical improvement. In 2 of the cases, where there was marked adenopathy, the glands were seen to shrink strikingly under therapy consisting of the glycolytic inhibitors. Parallel clinical improvement was noted in appetite, strength and loss of cough and itching. The results in one of the cases is illustrated in the following protocol.

J. F., 32 year old white female, admitted to the Brooklyn Cancer Institute on February 2, 1947 with a 2 year history of adenopathy starting after delivery of a normal child. The adenopathy had increased strikingly for the past 7 months, particularly in the left cervical region. Examination revealed extensive cervical adenopathy which surrounded the neck in a collarlike fashion, completely effacing the chin line. There were also extensive axillary adenopathy and hepatosplenomegaly. Chronic non-productive cough was present. X-ray revealed marked swelling of the left neck shoulder angle and considerable enlargement of both hilar, more prominent on the right. The upper mediastinum bulged to the right. W.B.C., 40,000 (polys 76 per cent), hemoglobin, 12.9 gm. Erythrocyte puncture revealed hyperplasia of the poly series, with no evidence of primary hematological disorder. The methylene blue reducing time, according to the method reported by one of us (M. M. B.), was 18.5 minutes (1).

After 3 weeks of treatment with the glycolytic inhibitors the glands receded markedly, the methylene blue reducing time dropped to normal and the W.B.C. decreased. Insensitivity to the medication occurred and was reversed by administration of sodium azide for a period of 1 to 2 weeks. This phenomenon of reversal of insensitivity has been accomplished 3 times thus far.

At present, about 6 months of therapy, the patient feels better, the glands are smaller and the cough is gone.

It is stressed that the agents used, and in the dosage employed, had no apparent effect upon normal cells. Thus in all cases of carcinoma treated with the same doses as the cases of leukemia there was no morphological change in any of the blood components. Similarly there was no apparent effect of inhibitor sodium fluoride on the clinical course or hematological picture in 4 cases of chronic myelogenous leukemia.
DISCUSSION

The therapeutic response observed, as well as the striking similarity between the calculated dose and the actual dose needed to produce therapeutic effects, lends support to the validity of the original thesis upon which these studies were undertaken.

It is also significant that the observations reported here give insight into the mechanism of adaptation by malignant cells. Although the phenomenon of adaptation still constitutes a major limitation in this and other chemotherapeutic modalities, it appears that it does not involve the creation of enzymatic mechanisms de novo but rather the utilization of previously existent secondary pathways. This would be in agreement with the studies of Sevag (20) with bacterial adaptation to chemotherapeutic agents. The adaptation found by us appears to follow a preferential and step-wise pattern. Thus the preferred pathway appears to involve the glycolytic cycle from the triose to lactic acid. On this basis the tricarboxylic acid cycle would serve as an accessory or secondary mechanism for energy production. Reactions involving azide-sensitive enzymes (cytochrome oxidase, peroxidase, catalase) may be considered as of limited importance as compared to their role in normal tissue. However, their functional potentiality is not absent but merely residual, and comes to prominence when more favored reactions are blocked. It should also be mentioned that some leukemic cells, which originally were similar in morphology to the adaptation cell described above, failed to show any sensitivity to the glycolytic inhibitors.

Correlation between biochemical activity and morphological alterations has long been a problem of acute interest to the cellular physiologist. Thus the apparent change in cell type in response to the glycolytic inhibitors poses new problems on the cytoplasmic localization of enzymes and the function of nucleoli. Much work is needed along these lines to clarify the full significance of these observations.

The studies reported by Stowell (20) on the relationship between nucleolar size and cytoplasmic nucleoprotein production in the liver cell is of great interest in this connection. He reported direct relation between nucleolar size and increase of cytoplasmic nucleoprotein.

The use of glycolytic inhibitors in the intact individual requires that particular attention be paid to the possibility of deleterious effects on the normal tissue of the retina and the intestinal mucosa, since both tissues have been reported as having metabolic activity similar to neoplastic tissue, particularly in regard to a high glycolytic activity. In our studies there have been no detectable impairment in vision, nor any change in gastrointestinal function or gross or microscopic alternation which would indicate appreciable damage. Either the metabolism of the intact retina is not similar to neoplastic tissue or it adapts very readily and with more facility than does malignant tissue.

The recent reports of Rosenthal (16) indicate that aerobic glycolysis of an intensity characteristic of cancer tissue is not a normal metabolic feature of the mucosa of the small intestine.

The need for further work with the therapeutic agents described is unquestioned. Of particular interest would be the incorporation of these simple molecules into the structure of a larger organic group so as to impart increased and more lasting effect. Thus arsenic, although toxic to the spirochete, requires a particular organic carrier to achieve maximal therapeutic and minimal toxic effect.

The data considered in this paper involve consideration of specific sensitivity of malignant tissue. However, the factor of the body substrate in the control or spread of tumors should not be overlooked. It is common clinical experience to find that similar tumors pursue widely different courses in different individuals. In this connection the variation induced in the reducing power of plasma of patients with malignant neoplastic disease is important. The beneficial therapeutic effect of sulfhydryl compounds reported by one of us (M. M. B.) (2) appears to be a valuable adjunct in the chemotherapeutic armamentarium. Further use of the sulfhydryl compounds in cancer patients has indicated the possibility of actual tumor inhibition and regression in addition to the relief of symptomatic complaints. These results would appear to be secondary to alteration or stimulation of body defense mechanisms rather than the result of direct effect on the tumor. The sulfhydryl compounds in present use include glutathione and cysteine. It appears advisable to extend observations to include compounds of di- and tri-thiol structure.

Study of the effects of the combined action of inhibitors and sulfhydryl compounds now in progress give indication of enhanced therapeutic effects.

SUMMARY

Differences in metabolism between normal and malignant tissues have been the basis for many attempts to develop chemotherapeutic measures. A new approach toward the chemotherapy of malignant tumors seems indicated in terms of the energy-rich phosphate bond.
Based on this concept, an hypothesis of biochemical function in malignant tissue as compared with normal was formulated as follows: With the advent of malignancy there is a significant alteration in the energy mechanism of tissue. The preferred pathways involve the glycolytic mechanisms while the respiratory enzyme reactions are diminished as compared with their role in normal tissue. The respiratory functional potentiality is not absent but merely residual. The glycolytic mechanism itself may be divided into (a) the primary portion from triose to lactic acid, and (b) the tricarboxylic acid cycle which serves as a secondary mechanism for energy production.

Based on this hypothesis the following were used in the treatment of various types of human malignant neoplastic diseases: sodium fluoride, iodoacetate, malonic acid and sodium azide. The essential validity of the foregoing hypothesis is indicated by the beneficial therapeutic effects observed in a significant number of patients treated.

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