NH₂ terminus of band 3 (53). In glycolysis 1 mole of glucose is converted into 2 moles of lactate. This ratio of almost 2:1 between lactate production and glucose consumption is always somewhat less than 2 since some of the glucose carbons are used for synthetic processes and do not pass through pyruvate kinase to pyruvate (54). Therefore cells without respiration need extracellular pyruvate to eliminate the cytosolic NADH produced by the glyceraldehyde3-phosphate dehydrogenase reaction and synthetic processes (55, 56). The pyruvate is excreted as lactate.

**Composition and function of blood**

Blood is a highly specialized tissue consisting of several types of cell suspended in a fluid medium called plasma. The cellular constituents consist of red blood cells (erythrocytes) that carry respiratory gases (O₂, CO₂). White blood cells (leucocytes) fight disease and platelets, cell fragments play an important part in the clotting of blood. Thus blood has a varied structure and performs wide range of functions. So far as transport is concerned, its two important constituents are the red blood cells (RBC) and plasma.

The prime function of red blood cells (RBC) is to carry oxygen from the respiratory organ to the tissues. There are approximately five million red blood cells per cubic millimeter of blood each being about 8 µm in diameter and 3 µm thick in the widest part. There is no nucleus in the mammalian blood cell and the cell is sunk in on each side giving it the shape of a biconcave disc. Surrounded by a thin elastic membrane, the whole of the interior of the cell is filled with the red pigmented protein, hemoglobin. The hemoglobin in RBC is the major functional protein responsible for carrying oxygen to different parts of the body. The red blood cell has a limited life span of about 120 days, and because of this the bone marrow manufactures new ones at the rate of about 1½ million/second to replace those destroyed.

The mature mammalian erythrocyte, for all of its physiological importance, has one of the least complicated biochemical organizations of the cell types. It has no nucleus, endoplasmic reticulum, or mitochondria, and it therefore unable to synthesize nucleic acids or proteins and lacks a kreb cycle and electron transport system. The erythrocyte is composed of two interdependent units: the membrane and the cytoplasm. The cytoplasm
contains hemoglobin as a major element and is equipped with all the enzymes of glycolysis. Despite its low energy production, the red cell can maintain itself for approximately 120 days. During this time it is subjected to numerous physical and chemical insults. The end result of all these factors is probably the slow depletion of enzymes and cofactors which cannot be replaced by the red cell (57).

CHART: SUMMARIZING THE CONSTITUENTS OF MAMMALIAN BLOOD
**Metabolism in red blood cell**

About 95% of the glucose consumed by red blood cells is metabolized via EMP (58). In essence, the pathway in erythrocytes is similar to that present in other tissues (59). The first series of reactions are involved in the phosphorylation of glucose to glucose 6-phosphate by hexokinase, isomerization of glucose-6-phosphate to fructose 6-phosphate by phosphohexose isomerase and a second phosphorylation of fructose to fructose 1,6-phosphate by phosphofructokinase. F 1, 6-phosphate by aldolase to two triose molecules, glyceraldehydes 3-phosphate and dihydroxyacetone phosphate. Unlike other tissues, the mature red blood cells normally contain insignificant levels of α-glycerol-phosphate (αGp) (60). This is consistent with the finding that these cells are apparently unable to synthesis lipid de novo (61). The conversion of DHAP to GAP is catalysed by triose isomerase with GAP undergoing the normal series of reactions to lactic acid. In the process of four ATP molecules are formed per molecule of glucose. Since two ATP molecules are expended to phosphorylate glucose to fructose 1,6-phosphate, a net of two ATP molecules is generated per molecule of glucose converted to lactic acid.

The rate of glycolysis in the human erythrocyte appears to be regulated by the coordinate action of a few rate-limiting enzymes. It was found that the enzymes hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) are appreciably displaced from equilibrium, suggesting that these steps are rate limiting.

The rate of glucose utilization in the red cell is primarily dependent upon the demands imposed upon the cell for energy. The fulfillment of such demands is an intricate process which involves the regulation of glycolysis by coordinating the action of the various rate-limiting steps.

Since the mature erythrocyte obtain energy solely from anaerobic glycolysis, its energy requirements assume a primary importance in the regulation of this pathway. Thus it is not surprising that the levels of ATP and Pi in the red cell are believed to exercise a regulatory influence on a number of rate limiting steps. A high level of ATP tends to decrease the rate of glycolysis by inhibiting the action of phosphofructokinase, phosphoglycerate kinase and pyruvate kinase (62). Such cells, which have a high ATP/ADP ratio, show a decrease in the rate of lactate production from glucose, an increase in the
levels of glucose 6-P and fructose 6-P, slight increases in fructose 1, 6-P, dihydroxyacetone phosphate and glyceraldehydes 3-P, and a decrease in the 3-PG, 2-PG and PEP.

Inorganic phosphorus exerts a dramatic effect on the activity of the EMP and it has been suggested that it is an important factor in its regulation (60). Increase in the concentration of the red cell results an increased rate of glucose utilization, ATP/ADP ratio and an accumulation of fructose 1, 6-P and triose phosphates (62).

Lactate dehydrogenase catalyze the reaction: pyruvate + NADH $\rightarrow$ lactate + NAD. It is one of the ubiquitous enzyme whose activity in serum is elevated in a considerable variety of disease states (63).

LDH (MW 134,000) has been extensively studied and many reviews have been written concerning it's multiple forms. There are two major types of LDH, each consisting of 4 identical subunits. One type is found predominantly in the heart (H) and other is in the skeletal muscle (M) are encoded by loci in chromosomes 11 and 12 respectively. Two subunits used in the formation of a tetramer yield five combinations: $H_4$(LDH-1), $H_3M$(LDH-2), $H_2M_2$ (LDH-3), $HM_3$(LDH-4), $M_4$(LDH-5). LDH isozymes can be separated by electrophoresis usually at pH 8.6. After separation, their location is determined by incubation of the supprot medium in a solution containing lactate and NAD$^+$. NADH generated at the LDH zones is detected by its reduction of a tetrazolium salt (nitroblue tetrazolium, NBT) to form an insoluble coloured complex via an intermediated radox carrier (e.g., phenazine methosulfate, PMS):

Diagramatic representation of the mechanism of enzyme activity stain of lactate dehydrogenase isoenzymes.

As long as the total activity of LDH is not very high so that the sustrate are not limiting, the intensity of the colour is approximately proportional to the various isozymes activities. Thus
densitometric scanning of an electropherogram provides an estimate of the activities of the individual enzymes as well as quantitation of their relative intensities. LDH 1 is the most negatively charged and fastest moving isoenzyme. LDH 5 is the least negatively charged and slowest migrating isoenzyme; the other isoenzymes possess intermediate mobilities. The normal relative intensity of LDH isoenzyme fractions is

$$LDH-1 < LDH-2 > LDH-3 > LDH-4 = > LDH-5$$

The mature human erythrocyte is a cell uniquely designed for one primary function. This primary function is to bring oxygen to the tissues in adequate quantities and to release it at partial pressure that are sufficient to permit its rapid diffusion from the blood to the metabolizing cells of the body. It is well known that glycolysis is the metabolic pathway best elucidated in many kinds of cells. A large part of the early discoveries of it's intermediate steps were made on red cells.
Experimental Procedure

Clinical samples

The subjects for the present study were selected from the patients admitted to the Calcutta Medical College, Kolkata with cervix cancer that required Radiotherapy. The patients were examined by gynecologists and referred to the hysterectomy or radiotherapy. Clinically two forms of cervical cancer have been found to apparently exist. A rarer fast growing type is being detected in all age groups and particularly in women in 20’s and 30’s. This form of cervical cancer is increasing at an alarming rate. The more common slowly growing form usually does not appear until 40’s, 50’s and 60’s. Its rate has remained relatively constant over the year. Early symptoms of cervical cancer include a watery or blood-tinged vaginal discharge and irregular or postcoital bleeding. Very early tumors often are occult stage, the tumor may appear ulcerated. Carcinomas developing in the endocervical canal may not be visible but cause the cervix to be probably enlarged and hard.

The study group comprised of total sixty five (n = 65) female patients of age group of 30-45 years. The control volunteer group consisted of non-smoking female individual without any gynecological problem or complication and blood sugar levels being normal. Written individual consent from all patients and control subjects were taken to carry out the present study. The clinical and biochemical details of the subjects are shown in Table 1.

Table 1. Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control</th>
<th>Cervical cancer(stage III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>35±2.9</td>
<td>42 ±3.2</td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
<td>24.6±2.3</td>
<td>18.6± 1.9</td>
</tr>
<tr>
<td>Blood Pressure (mm Hg)</td>
<td>120±5.5/ 75±3.2</td>
<td>95±6.2/ 65± 2.8</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>98.5± 4.3</td>
<td>90± 3.8</td>
</tr>
<tr>
<td>Haemoglobin(gm%)</td>
<td>12.6± 2.2</td>
<td>7.8± 1.8</td>
</tr>
<tr>
<td>No of issues</td>
<td>&lt; 3</td>
<td>&gt; 3</td>
</tr>
</tbody>
</table>
Collection and sampling

The syringe was first flushed with heparin (5000 IU in 5 ml). Then five millilitres (5 ml) of venous blood was obtained from each of the patients and also from the control group with the help of the doctors. Hemolysate of the red blood cell has been prepared according to the method of Yashida et. al (64).

Sample preparation

One millilitre (1 ml) of heparinised whole blood sample was taken in an eppendorf tube and centrifuge (in cold) at 2,500 x g for ten minutes at 4°C. The supernatant (plasma) thus obtained was separated. The remaining packed red cells were washed thrice with isotonic saline (0.9%) to remove any residual plasma and the buffy coat was discarded. The washed packed erythrocyte were lysed by adding chilled double distilled water to it and allow to stand for half an hour at 0°C-4°C. This is the osmotic shock method for lysing the cells. Then the suspension was ultracentrifuged at 13,000 x g for one hour at 2°C and cytosol was taken to as the enzymes of glycolytic pathway and HMP shunt pathway, antioxidant enzymes and LDH isozyme study.

All enzymes assayed were linked to the production or utilization of reduced nicotinamide adenosine dinucleotide (NADH) or its phosphate (NADPH) using U-2000 spectrophotometer. An extinction coefficient of 6.22 cm²/mol of 340 nm was assumed for the reduced coenzyme and used to calculate enzyme activity. A unit (U) was defined as the quantity of enzyme required to produce or consume 1 μmol of NAD(P)H per minute at 37°C. The enzyme activity was expressed μmol or nanomole per miligram of respected protein.

Statistical analysis

Results have been expressed as mean± SEM (standard error of mean). Statistical significant differences between values were obtained using the two-tail Student’s ‘t’ test. The values of significance were evaluated with ‘p’ values. The difference were considered highly significant at p, 0.001 and significant at 0.001< p< 0.01.
I. Enzymatic Analysis (Enzyme activity of glycolytic pathway)

Estimation of Hexokinase Activity (HK, EC 2.7.1.1)

Hexokinase (HK) activity was measured according to the method of Joshi and Jaganathan (65) based on the estimation of NADPH which is equivalent to the amount of glucose-6-phosphate produced by the reaction. The assay medium contained 3 ml of reaction mixture containing glucose (0.15 M), MgCl₂ (0.2 M), Tris HCl buffer (0.2M, pH 7.6), EDTA (0.0001 M), NADPH (0.0013 M) and glucose-6-phosphate dehydrogenase (2 units / ml). The reaction was initiated by the addition of 0.1 ml of enzymes and 0.1 ml of ATP (0.3 M, pH 7.6). The rate of change in optical density (OD) at 340 nm was followed at 1 minute interval for 5-6 minutes in U-2000 Hitachi spectrophotometer. The activity of HK has been expressed as μmoles of G-6-produced per minitic per milligram of protein.

Estimation of phosphoglucoisomerase activity (PGI, EC 5.3.1.9)

The phosphoglucoisomerase (PGI) activity was determined according to the method of Roe et al. modified by Buell et al. (66). The incubation media contained 90 mM glucose-6-phosphate; 0.1 M Tris-HCl buffer (pH 8.0) in a volume of 1 ml. The mixture was incubated for 10 minutes at 37°C. Then 2 ml of colour reagent (a fresh mixture of 40 volumes of 20N sulphuric acid with 1 volume of glacial acetic acid containing 0.4% resorcinol and 1% thiourea) was added, tapped vigorously in order to mix them. The colour was developed by heating the mixture in a water bath at 60°C for 20 min and reading were made at 500 nm. The specific activity of the enzyme has been expressed as μmoles of fructose 6-phosphate produced per minute per milligram of protein.

Estimation of phosphofructokinase (PEK, EC 2.7.1.11) activity

The phosphofructokinase activity was assayed following the combined method of Ling et al. (67). The incubation system contained 2 mM ATP solution, 33 mM Tris HCl buffer (pH 8.0), 5 mM MgSO₄, 0.05 M KCl, 0.1 M dithiothreitol, 2mM dipotassium fructose-6-phosphate in a volume of 2 ml. The mixture was incubated at 37°C for 30 minutes and the reaction was stopped by immersing the tubes in a boiling water bath for 5 minutes. After cooling 0.1 ml of aldolase (10 mg/ml) was added to the mixture as the auxiliary enzyme.
The mixture was incubated for 15 minutes at room temperature and was stopped by the addition of 1 ml ice cold 10% TCA. Then the mixture was centrifuged at 4,000 xg for 10 minutes. The aliquot was treated with 0.75 N NaOH for 10 minutes. Then 0.5 ml of 2,4-dinitrophenyl hydrazine (2,4-DNPH) was added. The mixture was incubated for 30 minutes and finally 3.5 ml of aqueous 0.75 N NaOH was added. The resultant product was measured at 540 nm. Each mole of fructose 1,6-phosphate produced by phosphofructo-kinase reaction leads to the formation of 2 mole of dihydroxyacetone phosphate. The specific activity of the enzyme has been expressed as μmoles of fructose 1,6-diphosphate produced per minute per milligram of protein.

**Estimation of Aldolase activity (EC 4.1.2.13)**

Aldolase activity was determined according to the colorimetric method of Beck (68). The assay system contained 0.1 M Tris-HCl buffer (pH 8.6), 0.05 M fructose 1,6-diphosphate, 0.56 M hydrazine buffer. The mixture was incubated for 10 minutes and then reaction stopped by addition of cold 10% TCA. It was then centrifuged at 4,000×g for 10 minutes and 1 ml aliquot was taken. The aliquot was mixed with 0.2 ml of 2,4-dinitrophenylhydrazine (2,4-DNPH) and incubated for 10 minutes at room temperature. The coloured triose phosphate hydrazone derivative was dissolved in 2.5 ml of 0.75 (N) aqueous NaOH. The specific activity has been expressed as μmoles of dihydroxyacetone phosphate produced per minute per milligram of protein.

**Estimation of Pyruvate Kinase Activity (PK, EC 2.7.1.40)**

The activity was measured following the method of Weber (69). The reaction mixture contained in a final volume of 3 ml 0.1 (M). Tris-HCl buffer (pH 7.4), 50 mM MgSO4, 100 mM KCl, 35 mM phosphoenolpyruvate, 1.5 mM adenosine diphosphate (ADP), 0.5 mM NADH, 0.1 ml lactate dehydrogenase (0.04 mg/ml), 0.1 ml of tissue homogenate at 37°C. The reaction was followed in a Hitachi spectrophotometer at 340 nm. The specific activity of this enzyme has been expressed as nmole of NADPH oxidized / min / mg of protein.
Estimation of Lactate dehydrogenase (LDH, EC 1.1.1.27)

The lactate dehydrogenase activity has been measured by the method of Nielands (70). The assay system contained 0.1 M glycine NaOH buffer (pH 10.0), 0.002 M NAD, 0.5 M DL-lactate in a volume of 3 ml. The reaction was followed at 340 nm in a Hitachi spectrophotometer at 1 minute interval for 5 to 6 minutes. The specific activity has been defined as nmole of NADH produced / min/ mg of protein.

II. Electrophoretic study for lactate dehydrogenase

(a) Gel electrophoresis: Polyacrylamide gel electrophoresis for LDH isozymes has been carried out according to the method of Brewer and Sing (71). Electrophoresis has been performed in gel apparatus with 10% polyacrylamide gels. The electrophoresis was carried out for 2 to 3 hrs at 4°C with a current 8 mA per lane from cathode to anode. 50 µg of enzyme preparation (in protein equivalent) conia (hemolysate) was charged in each lane.

(b) Staining procedure: LDH activity was detected in the acrylamide gel by suitable staining method. After electrophoresis the gel was incubated with freshly prepared solution containing 1.0 M lactate syrup, 10 mg/ml NAD, 0.1 M NaCl, 5 mM MgCl₂, 1 mg/ml nitroblue tetrazolium, 1 mg/ml phenazine methosulphate and 0.5 M phosphate buffer (pH 7.4) at 37°C for one and half hour in dark.

(c) Destaining: After visualization of LDH isozyme the reaction was stopped by immersing the stained gel in a 7.5% acetic acid solution. Finally the gel was prepared for densitometric scan. The relative proportion of each isozymes of LDH were evaluated by integrating densitometric (version 1.1) curves.
Results and Discussion

Glycolysis is ubiquitous, central metabolic pathway for glucose metabolism in the biosphere. It is found not only in all mammalian cells, but even in yeast and bacteria, due to the fact that it is present in most organisms, and also because it is the pathway by which, an important nutrient, glucose, is consumed. It is relatively inefficient bio-energetic pathway, recovering only about 5% of the energy available by oxidative metabolism of glucose in cells containing mitochondria. The glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), aldolase, phosphofructokinase (PFK), lactate dehydrogenase (LDH) and pyruvate kinase (PK) have been recently shown to assemble into a complex on the inner surface of the human erythrocyte membrane (72, 73).

Most cancer cells exhibit increased glycolysis and use this metabolic pathway for generation of ATP as a main source of their energy supply. This phenomenon is known as the Warburg effect and is considered as one of the most fundamental metabolic alterations during malignant transformation. In recent years, there are significant progresses in our understanding of the underlying mechanisms and the potential therapeutic implications. Biochemical and molecular studies suggest several possible mechanisms by which this metabolic alteration may evolve during cancer development. These mechanisms include mitochondrial defects and malfunction, adaptation to hypoxic tumor microenvironment, oncogenic signaling, and abnormal expression of metabolic enzymes. Importantly, the increased dependence of cancer cells on glycolytic pathway for ATP generation provides a biochemical basis for the design of therapeutic strategies to preferentially kill cancer cells by pharmacological inhibition of glycolysis. The glycolytic inhibitors are particularly effective against cancer cells with mitochondrial defects or under hypoxic conditions, which are frequently associated with cellular resistance to conventional anticancer drugs and radiation therapy. Because increased aerobic glycolysis is commonly seen in a wide spectrum of human cancers and hypoxia is present in most tumor microenvironment, development of novel glycolytic inhibitors as a new class of anticancer agents is likely to have broad therapeutic applications (74).

Study of enzyme activities during carcinogenesis as tumor markers has attracted attention (75, 76). Alteration in these enzymes activities occur during the early stage of malignancy and thus are detectable much before morphological changes. Far from being
completely understood, the regulation of this pathway witnessed several important progresses during the last few years. Glucose enters the RBC by facilitated diffusion, via the insulin-independent glucose transporter, GLUT-1. The glucose concentration in the RBC is not significantly different from that in plasma. Glycolysis proceeds through a series of phosphorylated intermediates, starting with the synthesis of glucose-6-phosphate (G6P). During this process, which involves 10 distinct enzymatically catalyzed steps, two molecules of ATP are expended (investment stage) to built up a symmetric intermediate, fructose 1,6-bisphosphate (F-1, 6-BP), which is then cleaved (splitting stage) to two three-carbon triose phosphatase into lactate. Two moles of ATP are formed from each mole of triose phosphate, yielding a net 2 moles of ATP per mole of glucose converted into lactate.

The present experiments were carried out to determine the specific activities of the different glycolytic enzymes in the erythrocyte of cervical cancer patient in order to investigate cancer induced alterations in the enzyme activity.

The first commitment of glucose to glycolysis is the phosphorylation of glucose to G-6-P, catalyzed by the enzyme hexokinase. The formation of G-6-P from free glucose and inorganic phosphate is energetically unfavourable, so that a molecule of ATP must be expanded in the phosphorylation reaction. Hexokinase has a low km (0.1mmol/L) for its substrates, and is normally saturated with both ATP and glucose. Among the enzymes on the main pathway of glycolysis HK is the pacemaker. It is observed that the specific activity of the hexokinase (Table 2 and Figure 1) in the erythrocyte of cervix cancer patient increased significantly. In this present study, there was no correlation between clinical staging or pathohistological grading of the tumor. The hexokinase activity is one of those that limit carbohydrate utilization (77), and its increase is advantageous for cell growth. Hexokinase never becomes lower than controls, in fact this would not agree with cell proliferation requirements.
Table 2. Specific activity of hexokinase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of hexokinase (nmol of G-6-P produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.86 ± 0.147</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>5.47 ± 0.535*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM for n = 20 subjects in each group. p values are calculated in comparison with respect to the control.

The conversion of G-6-P into F-6-P is catalyzed by phosphohexose isomerase, an enzyme widely distributed in animal tissues. It would probably be more accurately described as phosphogluco isomerase, since it differs in phosphomannose isomerase, the enzyme catalyzing the corresponding conversion of mannose-6-phosphate into glucose-6-phosphate. It has optimal activity at pH 9, but does not appear to require any activators or coenzymes. It is slightly inhibited by Mg$^{++}$ or Mn$^{++}$ and by 6-phosphogluconic acid. Phosphohexose isomerase activity in erythrocyte is 100 times high relative to that of the serum. The result shows (Table 3 and Figure 2) decreased activity in cervical cancer.

Table 3. Specific activity of phosphogluco-isomerase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of phosphogluco-isomerase (nmol of F-6-P produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.68 ± 0.496</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>5.83 ± 0.416*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM for twenty subjects in each group. p values are calculated in comparison with respect to the control.

Parr (78) suggested that phosphohexose isomerase can be modulated in vivo by 6-phosphogluconate, a powerful glucose 6-phosphate isomerase competitive inhibitor, which should accumulate when 6-phosphogluconate dehydrogenase activity is lower than that of glucose 6-phosphate dehydrogenase.
Glucose 6-phosphate dehydrogenase, glucose 6-phosphate isomerase, 6-phosphogluconate dehydrogenase activities require particular attention due to the strategic position in the key branch point of glucose 6-phosphate partitioning between glycolysis and pentose phosphate cycle. Glucose 6-phosphate isomerase is not a limiting factor for glucose 6-phosphate channeling into glycolysis; this finding also agrees with neoplastic cell behavior, which is largely glycolysis dependent (79-81). Phosphofructokinase is a complex regulatory enzyme (82). Binding of phosphofructokinase to the erythrocyte membrane in vitro relieves allosteric inhibition by ATP and 2, 3 bisphosphoglycerate, converting the sigmoidal fructose 6-phosphate saturation curve to a hyperbolic form (83). Phosphofructokinase (PFK-1) has the dominant regulatory role in glycolysis, controlling the flux of F-6-P through glycolysis and indirectly the level of G-6-P and inhibition of hexokinase. Although present at several times higher concentration than hexokinase, PFK-1 activity is uniquely sensitive to the energy status of the cell. ATP is both a substrate and an allosteric inhibitor of PFK-1, exerting fine control over the activity of the enzyme. It showed increased activity in cancer (Table 4 and Figure 3) and it has a reciprocal interaction with fructose-6-phosphate and magnesium (MgATP\(^2\)).

Table 4. Specific activity of phosphofructokinase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of phosphofructokinase (μmoles of F 1,6-P produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.428 ± 0.007</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>1.216 ± 0.072*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM for twenty subjects in each group. p values are calculated in comparison with respect to the control.

Several enzymes playing parts in the aerobic metabolism of glucose have been found to be of diagnostic importance and a landmark in the biochemical investigation of malignant diseases. Aldolase is highly specific for fructose 1,6-phosphate, but while it shows slight activity against fructose 6-phosphate, it is virtually inactive with fructose-1-phosphate as substrate. The result shows (Table 5 and Figure 4) increased activity in cervical cancer patient.
Table 5. Specific activity of aldolase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of aldolase (µmoles of DHAP produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.44 ± 0.057</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>2.50 ± 0.206*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM for twenty subjects in control group and twenty four in diseased group. p values are calculated in comparison with respect to the control.

Pyruvate kinase is responsible for the transfer of phosphate from phosphoenol-pyruvate to ADP accompanied by formation of ATP and pyruvate. One key sensor of this regulation is the glycolytic enzyme pyruvate kinase. Phospho-enolpyruvate, the substrate of pyruvate kinase is the most energy rich cellular phosphometabolite. Therefore, the pyruvate kinase reaction strongly favors the formation of ATP. It showed increased activity (Table 6 and Figure 5) in cancer.

Table 6. Specific activity of pyruvate kinase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of pyruvate kinase (µmoles of pyruvate produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.125 ± 0.175</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>2.690 ± 0.120*</td>
</tr>
</tbody>
</table>

Specific activity of pyruvate kinase in the hemolysate of cervical cancer patients and healthy controls. Results are expressed as mean± SEM for twenty subjects in each group. p values are calculated in comparison with respect to the control.

Lactate dehydrogenase (LDH) is a widely distributed Zn²⁺ containing enzyme, reversibly converts lactate into pyruvate in the presence of NAD. Raised level is usually associated (Table 7 and Figure 6) with widely dissimilated manifestations of malignant disease, but in certain cases normal levels may be found even when the presence of extensive metastases is clearly demonstrable.
Table 7. Specific activity of lactate dehydrogenase in hemolysate (mean± SEM)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Specific activity of lactate dehydrogenase (nmol of lactate produced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.940 ± 0.062</td>
</tr>
<tr>
<td>Cervix Cancer</td>
<td>2.100 ± 0.232*</td>
</tr>
</tbody>
</table>

Table shows specific activity of lactate dehydrogenase in the hemolysate of cervical cancer patients and healthy controls. Results are expressed as mean± SEM for twenty subjects in each group. p values are calculated in comparison with respect to the control.

In this study the main interest is focused on the clarification of the interplay of the constituent reactions of a metabolic pathway. Several distinct isozymic systems which were observed to have alterations during differentiation from normal to the neoplastic state have been reviewed. Most of these isozymes are key pathway or regulatory enzymes of intermediary metabolism, lactate dehydrogenase has been extensively studied. Isozymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different kinetic property.

Shifts in the LDH isozyme patterns have been observed during development and under changing biological conditions. LDH isozyme shifts are frequently observed in cancers. Increased levels of the M subunit or the M form of the LDH (isozyme 3 and 5) have been observed (Figure 7) in cervical cancer where LDH 1 is considered as loading control. Lactate dehydrogenase isozyme shifts are frequently observed in cancers (84). Dawson (85) found an absolute increase in lactate dehydrogenase activity and an increase in M subunits upon examination of several human tumors. Increased levels of the M subunits or the M form of lactate dehydrogenase have been observed in breast tumor (86), uterine carcinoma (87), malignant colon (88), a series of carcinomas (89) and Morris "minimal-deviation" hepatomas (90). It would appear that the lactate dehydrogenase in tumors is more like the muscle enzyme, which is the major form found in tissues exhibiting a high rate of glycolysis and is not inhibited by pyruvate.
Figure 7 shows the isoenzyme pattern of lactate dehydrogenase. Isoenzymes are obtained by polyacrylamide gel electrophoresis of membrane proteins from red blood cell using 10% gel stained with enzyme activity stain. Lane A, Lane B, Lane E contains the membrane proteins of cervical cancer patients. Lane C and Lane D contains the membrane proteins from suspected cervical cancer patients and Lane F contains membrane proteins from control (healthy volunteers). 50 µl of hemolysate was loaded in three different groups. LDH-1 is marked as the loading control. The relative intensity of LDH 1 is same in all the cases. In every case the relative proportion of the LDH-3 and LDH-5 are normalized by loading control. LDH 3 and LDH 5 are found to be overexpressed in case of cervical cancer compared to control.