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Glucose Metabolism in Normal and Virus-infected Chick Cells in Culture

Abstract. By using two-dimensional paper chromatography combined with autoradiography, it is possible to determine the quantitative differences in patterns of glucose metabolism in animal tissue culture cells under various conditions. The similarity of pattern of normal cells in high glucose to that of virus-transformed cultures suggests some similarity in underlying control mechanisms involved in glucose metabolism.

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It is generally accepted that malignant cells are more "glycolytic" than their normal counterparts (1), although the extent and reason for such a difference has been the subject of many controversies (2). Bissell et al. (3) have demonstrated that the increased lactate production after infection of chick cells with Rous sarcoma virus (RSV) cannot be accounted for by increased growth rate as previously suggested (4). We now report the isolation and quantitative determination of intermediates in glucose metabolism which confirm and expand the above finding. In addition, the technique employed here provides a sensitive tool for studying the overall metabolic pattern of animal cells in tissue culture.

Primary cultures were prepared from 10-day old chick embryos as previously described (5). Cells were seeded in medium 199 supplemented with 2 percent tryptose phosphate broth, 1 percent calf serum and 1 percent heated chicken serum. Secondary cultures were prepared 4 days after the primary seeding by trypsinization of primary cultures and were seeded at 1×10^6 cells per 35 mm dish. For studies with transformed cultures, half the cells of a single embryo were infected 0-4 hours after primary seeding with either Bryan or the Schmidt-Ruppin strain of Rous sarcoma virus as previously described (3).

After 48 hours, the secondary cultures were washed 3 times with glucose-free Hank's buffer. Medium containing uniformly labeled glucose and/or ^{32}P was added to each culture. The cells were permitted to take up the radioactive substrates for an hour. The medium was then removed, the cells were washed rapidly with cold unlabeled glucose-containing Hank's buffer, and killed by addition of 80 percent methanol.

The killed cells were then scraped with a rubber policeman, disrupted by sonic oscillation, and applied to filter paper for analysis by two-dimensional paper chromatography (6). Locations of the labeled metabolites were detected by radioautograms and the content of ^{14}C was determined (6). The unknown spots were eluted and the procedure was repeated after addition of unlabeled known compounds which later were localized with appropriate chemical reactions.

The rate of incorporation of ^{3}H[thymidine] into DNA was used as a measure of overall rate of DNA synthesis, and the rate of glucose uptake was measured by using 2-deoxyglucose (3,7).

A comparison of the metabolic pattern of normal and transformed cells (Fig. 1A) demonstrates that in transformed cultures the ratio of the intracellular pool of lactate (as a measure of the glycolytic pathway) over citrate (as a measure of the TCA cycle) is increased appreciably. Labeled pool sizes of other intermediates of the TCA cycle and those derived from it (malate, glutamate and aspartate shown in the chromatograms) are decreased. The labeled pools of the sugar diphosphate region (DIP), the triose phosphate intermediates (3PGA and DHAP) and the metabolites of the oxidative pentose phosphate pathway (PMP; "hexose monophosphate shunt") are increased measurably. An increased utilization of oxidative pentose phosphate pathway over TCA cycle after transformation was previously observed using CO_2 derived from glucose labeled in 1 or 6 positions (3).

It is usually reported that transport and phosphorylation of glucose are coupled processes and that there is very little or no free glucose inside the cells (8). The presence of a relatively large glucose pool (Gluc) in these chromatograms was therefore surprising. Since the amount of glucose

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in the medium relative to that of label in the cells is enormous, part or all of the glucose pool could be due to contamination from the medium, despite the extensive washing. However, from this and other experiments, we believe that the possibility that a free glucose pool does exist within these cells cannot be ruled out. It is possible that the hexokinase step is not necessarily coupled to glucose transport (see also ref. 13). This problem and the identification of the remaining spots of the chromatograms are under investigation.

To determine the contribution of the faster growth rate of transformed cells to these patterns, 5 percent chicken serum was added to normal cells 16 hours prior to the onset of the experiment. Serum causes the normal density-inhibited cells to undergo rapid proliferation (9). While addition of serum increases the glycolytic degradation of glucose, the ratio of labeled lactate to citrate is still at least 3 times lower than in transformed cultures (Fig. 1B and Table 1). Moreover, the pattern attributable to added serum seems to be due to factors other than growth alone: addition of 5 percent serum to transformed cultures also increases the lactate to citrate ratio without changing the growth rate appreciably (Table 1). Growth per se, therefore, cannot explain the changes observed.

The suppression of respiration and the increased glycolysis by glucose was first described by Crabtree in 1929 (10). When the normal cells receive decreasing levels of glucose, the ratio of lactate to citrate decreases proportional to the level of glucose in the medium. A 10-fold change in the level of glucose concentration of the medium causes a 15-fold change in the ratio of lactate to citrate (Fig. 2A, Table 2). Addition of serum to both cultures raises the background level of glycolysis, yet a substantial

difference between cells in high and low glucose remains (Fig. 2B and Table 2). The labeled pools of the DIP, PMP, and triose phosphate regions are also increased with increased glucose concentration.

It is known that transformed cultures transport glucose at a faster rate than normal cells (11,12,3,7) [this has been shown to be a reflection of a change in V_{max} for transport of glucose in cells transformed by both RNA and DNA viruses (13) rather than a change in the affinity of the transport system for glucose as reported earlier (8)]. We therefore suggest that the increased glucose (or glucose-6-phosphate) concentration within the transformed cells creates a situation similar to that of normal cells in high glucose medium and causes the shift to a higher glycolytic metabolism, i.e., the Crabtree effect, and increased glycolysis in transformed cultures may have a similar underlying mechanism. The absolute levels of glycolysis and respiration change from embryo to embryo, with population density (3) with changes in growth condition. The glucose level within the cell is thus only one of the factors causing the observed balance between glycolysis and respiration. But other factors being equal, whatever increases the glucose entry into the cell seems to cause an inhibition of respiration and an increase in glycolysis whether it is addition of serum, an increase in glucose concentration of the medium, or viral transformation.

The techniques employed in this study allow determination of the various pool sizes of metabolites during a condition of steady-state metabolism. This information, together with knowledge gained from examination of changes accompanying perturbation of the steady-states will permit the elucidation of regulatory steps. Such a procedure has been

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employed successfully with photosynthetic cells (14). Changes in glucose metabolism due to pH, serum and hormone addition, cell density and various inhibitors may be effectively examined in this way. For example, preliminary kinetic experiments towards this end indicate a rapid rise in the F-1,6-Dip/F6P ratios (see also Tables 1 and 2) suggesting the phosphofructokinase step as a controlling point in such transitions.

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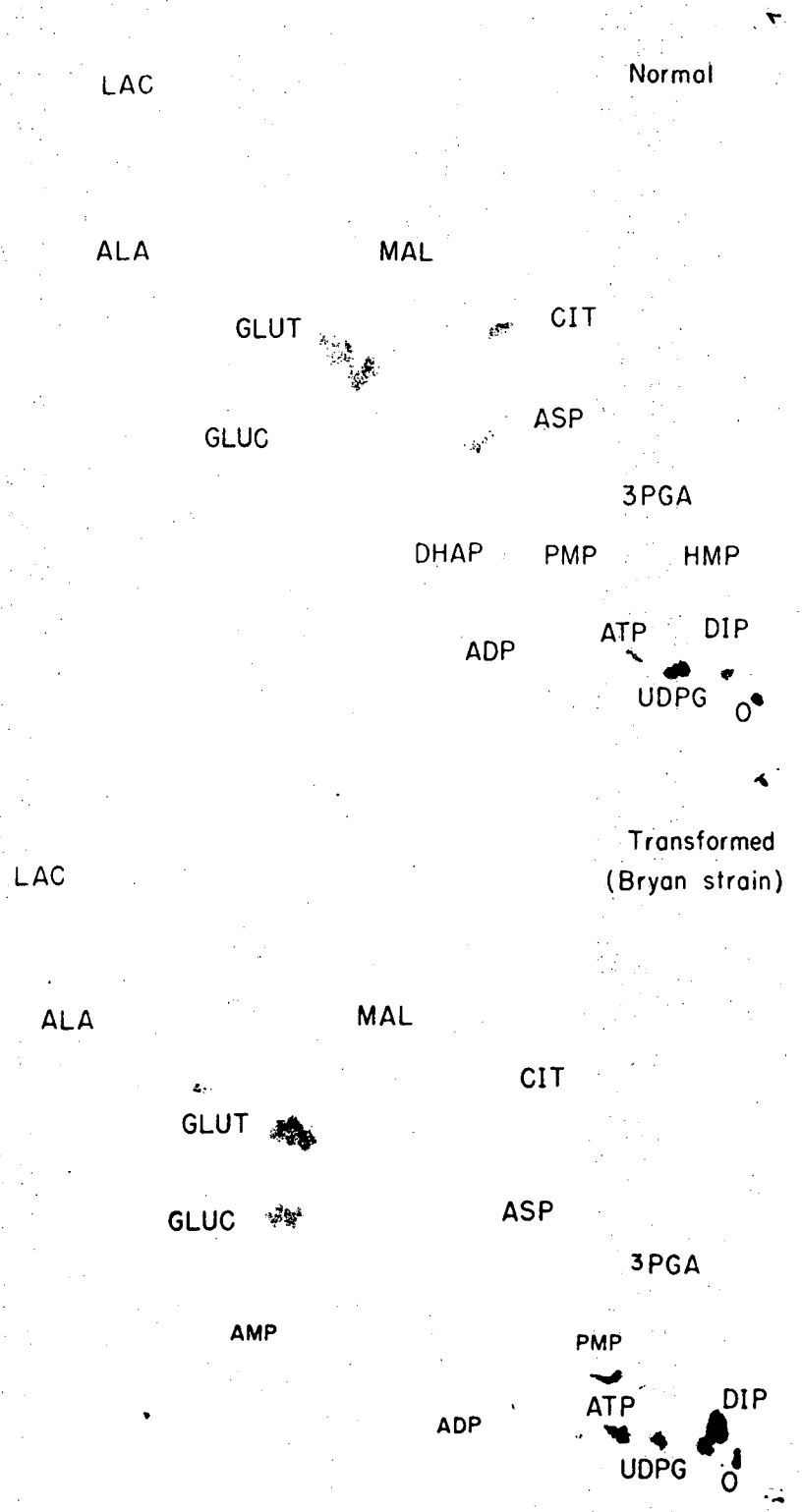
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MAL
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DHAP
PMP
HMP
ADP
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Transformed
(Bryan strain)
LAC
ALA
MAL
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GLUT
GLUC
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AMP
PMP
ATP
DIP
ADP
UDPG
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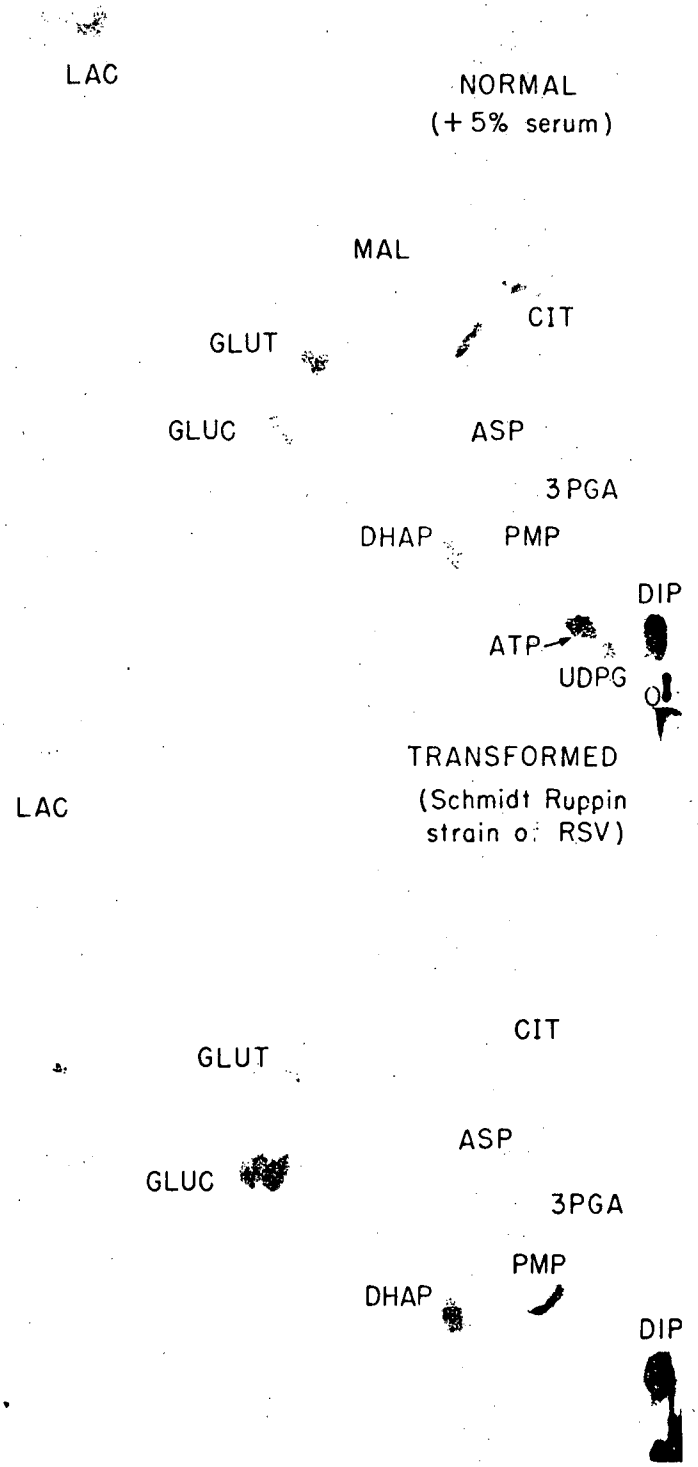
Fig. 1a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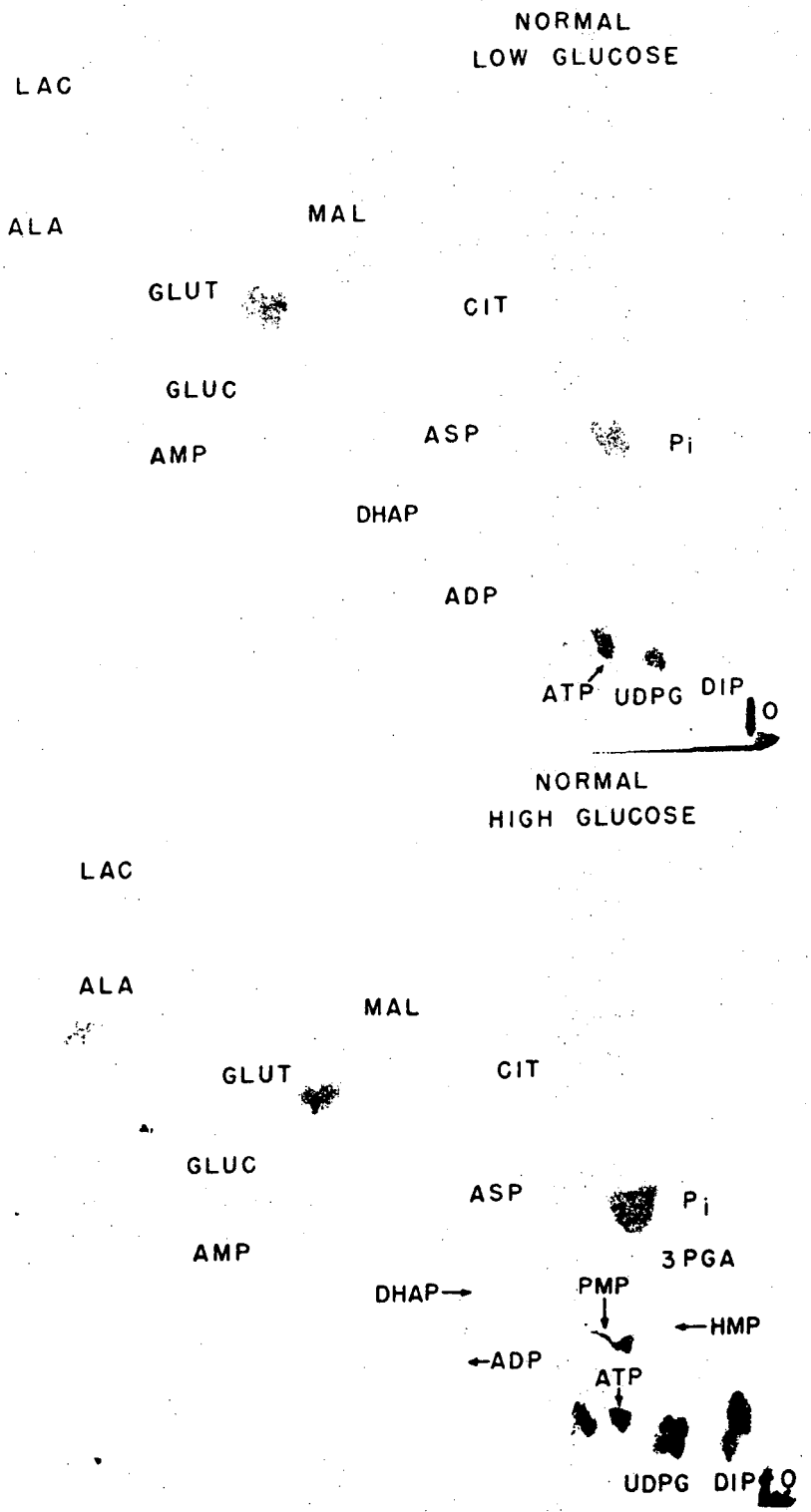
(+5% serum)
MAL
CIT
GLUT
GLUC
ASP
3PGA
DHAP
PMP
DIP
ATP
UDPG
TRANSFORMED
(Schmidt Ruppin
strain of RSV)
LAC
CIT
GLUT
ASP
GLUC
3PGA
PMP
DHAP
DIP

Fig. 1b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CIT
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ASP
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NORMAL
HIGH GLUCOSE
LAC
ALA
MAL
GLUT
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Pi
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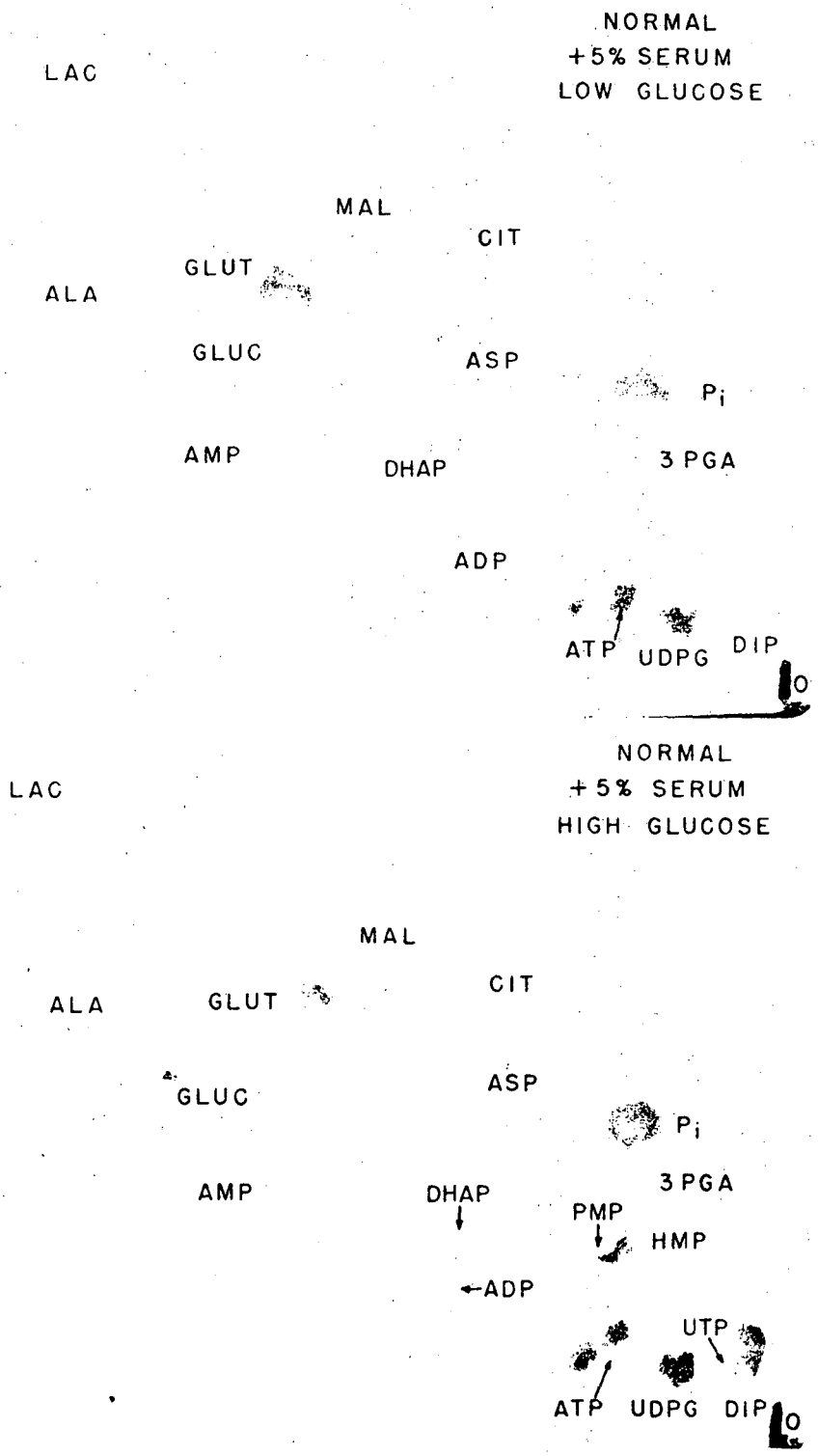
Fig. 2a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LOW GLUCOSE
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MAL
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Fig. 2b

FIGURE LEGENDS

Fig. 1. Pattern of glucose metabolism in normal and Rous transformed cells. The cells were exposed to 0.75 ml of 5.5 mM uniformly labeled glucose (final sp. act. 214 mc/mM, New England Nuclear) in medium 199 for one hour. After removal of medium and washing, the methanol extracted pools together with sonicated cells were applied to paper. The samples were first eluted with phenol:water:acetic acid (840:160:10) for 24 hours. After drying, the paper was turned 90° and run with butanol:water:propionic acid (500:280:220) for another 24 hours. The radioautograms were developed after one week of exposure to the paper. Abbreviations used: Gluc, glucose; Glut, glutamate; Asp, aspartate; Malate, malate; Lac, lactate; Ala, alanine, DHAP, dihydroxyacetone phosphate; 3PGA, 3-phosphoglyceric acid; DIP, sugar diphosphate region; PMP, pentose monophosphate region; HMP, hexose monophosphate region; UDPG, uridine diphosphate glucose; O, origin.

Fig. 2. Pattern of glucose metabolism in normal chick cells in low and high glucose. Procedure as in Fig. 1. The cultures also received ^{32}P (final sp. act. 30 μc/μM, New England Nuclear) concomittantly with glucose. During the labeling the phosphate concentration of the medium was 10 x less than the experiment reported on Fig. 1. Pi, inorganic phosphate.

Table 1. Integration of glycolysis and respiration in normal and virus-transformed cells.

	$\frac{\text{lactate}}{\text{citrate}}$	$\frac{\text{F-1,6-DiP}}{\text{F6P}}$	3H[Tdr] incorp. into DNA $\frac{\text{CPM}}{\mu\text{g protein}}$
Normal	2.0	3.9	19.3
Transformed	20.0	8.2	68.0
Normal + Serum	5.6	7.8	51.0
Transformed + Serum	32.5	13.1	73.8

This is the average of 2 experiments; the procedure was as in Fig. 1. The spots were removed from chromatograms and counted (6). The DIP and HMP regions were eluted with 0.3-0.5 ml water, treated with phosphatase [purified from Polidase S (Schwarz Laboratories) by ammonium sulfate precipitation; 25 µg/0.3 ml, for 3 hours at 37°C] and re-chromatographed as previously. The isolated fructose diphosphate (F-1,6DiP) and fructose-6-phosphate (F6P) were removed and counted. Last column indicates the rate of DNA synthesis as measured by ^{3}H-thymidine incorporation. Glucose concentration was 5.5 mM.

Table 2. Crabtree effect in normal chick-embryo fibroblasts.

		$\frac{\text{lactate}}{\text{citrate}}$	$\frac{\text{F-1,6-DiP}}{\text{F6P}}$
Normal cells	0.55 mM glucose	1.8	4.9
	5.5 mM glucose	28.0	8.5
Normal cells + 5% serum	0.55 mM glucose	5.2	11.3
	5.5 mM glucose	43.5	19.0

Legend as in Fig. 2 and Table 1.

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