Effect of Glucose and Galactose on Microcirculatory Flow in Normal and Neoplastic Tissues in Rabbits

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ABSTRACT

Red blood cell (RBC) velocity and vessel lumen diameter were measured in individual microvessels of both normal (mature granulation) and neoplastic (VX2 carcinoma) tissues grown in a transparent ear chamber in anesthetized rabbits. RBC velocity was measured using the dual-slit photometric technique and the diameter of the vessels was determined using an image shearing device. From these measurements, the mean volumetric flow rates in the individual vessels were determined. Glucose produced a 90% decrease in RBC velocity and blood flow rate in the tumor at 65 min postinjection without modifying the flow in the normal tissue. Galactose, on the other hand, decreased RBC velocity and blood flow in the tumor and normal tissues by approximately 90 and 55%, respectively, at 65 and 5 min postinjection. Neither glucose nor galactose caused any statistically significant changes in vessel diameter, WBC adhesion to vessels, microvascular permeability, or systemic viscosity.

INTRODUCTION

A number of investigators have shown that hyperglycemia leads to reduction in tumor blood flow and pH (1-10). These studies suggest that the tumor pH reduction is a result of both conversion of glucose into lactic acid and decreased egress of lactic acid caused by reduction of tumor blood flow due to glucose (9). Further, DiPette et al. (10) have found that blood flow reduction in tumors is a result of both systemic and local changes caused by glucose. Whereas the systemic changes have been shown to include reduction and redistribution of the cardiac output. Local mechanisms for glucose include blockage of rigid RBC in the tortuous vasculature of tumor and increase in local blood viscosity also due to RBC rigidity. Increase in RBC rigidity is caused by glucose itself as well as by low pH in tumors. Results also show that the local mechanisms responsible for blood flow reduction following galactose injection differ from those responsible for the reduction following glucose injection.

MATERIALS AND METHODS

Preparation of the Rabbit Ear Chamber. Sandison-type rabbit ear chambers (One of a Kind, Ltd., Lincoln Park, NJ) were implanted into the right ear of anesthetized (25 mg/kg Nembutal, i.p.) male New Zealand White rabbits (Hilltop Laboratories, Scottsdale, PA) following the procedure described in Ref. 11. These chambers consist of six windows which enable the formation of thin regenerated tissue beds (40 ± 10 μm), thus allowing in vivo observation of the microvasculature. The chamber was matured 40-50 days after implantation (11) of the chamber and at this time the chamber was used either for normal tissue studies or for tumor implantation.

Tumor Implantation. VX2 carcinoma was originally obtained from Dr. J. A. Dickson, Cancer Research Unit, Department of Biochemistry, University of Newcastle-Upon-Tyne, England, and is maintained in our laboratory by biweekly i.m. injections of 0.1 ml of tumor slurry into the flank of New Zealand rabbits. This tumor is a highly malignant, anaplastic, squamous cell carcinoma that originated from a spontaneous transformation of a virus-induced skin papilloma in a domestic rabbit over 45 years ago (13).

To implant the tumor in the chamber, the animal with the tumor was anesthetized (Nembutal, 25 mg/kg i.p.) and the coverslip protecting the mature granulation tissue was replaced with another coverslip coated with a slurry of approximately 10^7 (10% viable) tumor cells. The neoplastic tissue was ready for microvascular study following 8-10 days of growth. Histological examinations were performed to characterize the neoplastic tissue (14).

Microscopic Blood Flow Measurements. RBC velocity and lumen diameter were measured both online and offline as shown schematically in Fig. 1. Once the normal or neoplastic tissue reached the desired stage of growth, the anesthetized rabbit (Nembutal, 25 mg/kg i.p.) was placed on a restraining board and the ear containing the chamber was

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2 Recipient of a NIH Predoctoral Training Grant (1981-1985) during the course of this work.

3 Recipient of the NIH Research Career Development Award (1980-1985) during the course of this work. To whom requests for reprints should be addressed.

Fig. 1. Schematic of experimental setup used to obtain microvascular parameters (online, - - - - -; offline, - - - - -). The setup permits RBC velocity and diameter measurements in more than one vessel in the chamber [modified from Dudar and Jain (12)].

secured to an x-y mechanical stage of a microscope. The tissue was transilluminated by a 100-W tungsten-halogen lamp (Carl Zeiss, Inc., Oberkochen, West Germany) driven by a stabilized DC power supply (Model 6263b; Hewlett Packard, Palo Alto, CA).

A section of the chamber was selected so that a well perfused vessel could be monitored for online measurements and 2–3 surrounding vessels could be recorded for later offline analyses. Once this position was determined, the chamber was locked in place for the duration of the experiment. For online measurements, the selected vessel was displayed on a video monitor (Model EVM-11; Electrohome, Ltd., Kitchener, Ontario, Canada) via a low-light video camera (Model 4410/SIT; Cohu, Inc., San Diego, CA). For offline measurements, the vessels were displayed on a second video monitor (Model EVM-11) via a high-frame-rate video camera (Model 4410A/SIT; Cohu) and also recorded on a video cassette recorder (Model NV-8500; Panasonic). Hence, this setup permitted data analysis in a number of vessels within the same chamber.

RBC velocity and diameter were measured in the online vessel for 5 min to obtain base-line values for these parameters. The other vessels were recorded on video tape. After 5 min, glucose or galactose (6 g/kg) was injected i.v. into the opposite ear and the parameters were measured for the remaining 1.75 h. The diameters of individual vessels were measured using an image shearing device (Model 907; IPM, Inc., San Diego, CA) during both online and offline analyses. The RBC centerline velocity was measured online with a fiberoptic photometer (IPM, Inc.) and offline with a video photoanalyzer (Model 2041H; IPM, Inc.) coupled to a velocity correlator (Model 102; IPM, Inc.). The voltages from the image shearing device and the velocity correlator were stored on a personal computer (IBM XT) via a data acquisition unit (Autodata Nine; Accurex, Inc., Mountain View, CA). Calibration functions previously determined (11) were used to convert these voltages to the desired velocity ($v_{\text{centerline}}$) and diameter (d) values.

The mean volumetric flow rate ($Q_{\text{mean}}$) for each vessel was then calculated as

$$Q_{\text{mean}} = 0.491\, v_{\text{centerline}}\, d^2(d > 8\, \mu m) \quad (A)$$

This equation was determined empirically and takes into account the effect of RBC deformability, plasma back leakage, and also the difference in flow velocity between the plasma and RBC (15). Note that the relationship between $v_{\text{centerline}}$ and $Q_{\text{mean}}$ depends strongly on the RBC radial velocity distribution. Clearly, a modification in the velocity profile was produced in our glucose experiments due to an increase in RBC rigidity and a decrease in the plasma skimming layer (see "Discussion"). Therefore, the relationship between $v_{\text{centerline}}$ and $Q_{\text{mean}}$ may vary at this time. Since a more accurate equation for calculation of $Q_{\text{mean}}$ from $v_{\text{centerline}}$ is not available in the literature, we used Equation A.

Due to the fact that anesthesia affects the blood flow (16) data were also collected for 2 h following the injection of Nembutal and saline (8 ml/kg i.v.). The results from these control experiments were compared to those following glucose/galactose injection to determine the effect of these agents on the blood flow. All experiments were started at 40 min after the anesthesia injection to normalize the data with respect to this time.

In separate experiments, glucose (6 g/kg) was injected i.v. into the ears of tumor-bearing New Zealand White rabbits and blood was withdrawn from the femoral artery via a catheter at 0, 25, 80, and 120 min postinjection. The blood samples were analyzed for blood glucose concentration using spectrophotometry and glucose oxidase kits (Bio-Dynamics, Indianapolis, IN) to determine the hyperglycemia level. The control levels were also determined following saline injection (8 ml/kg i.v.).

WBC Adhesion. To study modifications in WBC adhesion in individual vessels following glucose and galactose injection, the rabbit ear chamber technique was used. Sandison-type rabbit ear chambers were implanted as described previously and VX2 carcinoma was implanted in the chamber to study the response in tumor vessels. In order to visualize the leukocytes in the microvasculature of the rabbit ear, light microscopy was used. The same optical system used for the blood flow measurements was used in this study (Fig. 1).

Once the normal or neoplastic tissue reached the desired stage of growth, the New Zealand White rabbit was anesthetized (Nembutal, 25 mg/kg i.p.) and secured to a restraining board. The ear containing the chamber was secured to the x-y mechanical stage and the chamber was positioned such that the vessel of interest was displayed on the video monitor. The image of the vessel was recorded for 5 min to obtain the base-line value for adhesion. At this time, glucose or galactose was injected i.v. (6 g/kg) into the opposite ear via a catheter and the image was recorded for another 1 h and 20 min. All experiments were started 40 min after the anesthesia injection to normalize the data with respect to this time and hence keep it consistent with the blood flow data.

Once the image was recorded, both qualitative and quantitative analyses were performed. WBC adhesion was quantified by counting the number of WBC that interacted with the vessel wall for periods longer than 0.5 s over a 5-min period. These measurements were performed every 10 min for the duration of the experiment. The vessels were also observed for any abnormalities such as an excess of WBC adhering in one area of the vessel and blocking flow, and WBC adhering for long periods of time. The diameter of the vessel was also measured prior to WBC count using the image shearing monitor.

Systemic Blood Viscosity. To measure modifications in systemic blood viscosity due to glucose and galactose injection, tumor-bearing New Zealand White rabbits were anesthetized (Nembutal, 25 mg/kg i.p.) and a polyethylene catheter filled with heparinized saline was inserted into the femoral artery. This catheter was used for blood sampling. A blood sample (2 ml) was taken via syringe prior to injection and was placed in a 2-ml Vacutainer blood collection tube containing 0.04 ml of 7.5% EDTA to prevent coagulation (Becton Dickinson; Rutherford, NJ). Then 0.5 ml of the blood was removed from the tube and used to obtain base-line values for whole blood viscosity at shear rates ranging from 2.25 to 225 s⁻¹. Five min following the determination of base-line values, glucose or galactose (6 g/kg) was injected i.v. via a catheter located in the ear. Blood samples were then taken 20, 75, and 115 min following injection and blood viscosity was determined. The viscosity was measured using a Wells-Brookfield cone/plate viscometer (Dial Reading LVT; Brookfield Engineering Laboratories, Inc., Stoughton, MA). Since viscosity is a function of hematocrit, hematocrits of the samples were determined using nonheparinized microcapillary tubes (Fisher Scientific, Pittsburgh, PA) and a microcapillary reader (Damon/ IEC Division; Needham Heights, MA).
experiments, the concentration ranged from 175.75 mg to 187.13 mg/100 ml and there was no significant change in concentration for the duration of the experiment. Following glucose injection, the blood glucose concentration was 1268 mg/100 ml 25 min postinjection and then decreased to 533 mg/100 ml in 2 h. These results clearly indicate that hyperglycemia was present in the system during the total duration of the glucose experiments.

Microvascular Response

Normal Tissue. The results obtained on diameter, RBC velocity, and volumetric flow rate for granulation tissue are shown in Table 1 (N = 15 vessels). In order to compare the results obtained following glucose and galactose injection with those obtained in the control experiments, the average values for each parameter were normalized with respect to the base-line values (i.e., at time = 0 min) and plotted as a function of time. These results are shown in Fig. 3. Statistical analysis was performed on these data using two different methods. A paired t test was performed on the absolute values of each parameter to compare each value with its base-line value (17). Significant modifications at a 95% confidence limit are represented as Footnote b in Table 1. Also, an unpaired t test was performed on the normalized values to determine if there was a significant difference between the glucose/galactose results and the control results. Significant differences between these values at a 95% confidence limit are represented by asterisks in Fig. 3.

The results in Table 1 and Fig. 3, left, indicate that there was no significant change in vessel diameter either in the control experiments or following glucose and galactose injection. The average diameters of the vessels used in these studies were 33.28 ± 0.45 (SE), 30.66 ± 1.87, and 34.82 ± 1.97 for the control, glucose, and galactose experiments, respectively. Following saline injection, the velocity slightly decreased at 50 min. A significant decrease (44%) was observed at 70 min and the velocity continued to decrease, reaching a value 83% below the base line at 110 min. Following glucose injection, a similar trend was observed. The velocity decreased by 37% at 70 min and continued to decrease throughout the remainder of the experiment. When comparing the normalized values obtained from the glucose experiments with those from the control experiments, no significant difference was observed (Fig. 3, middle). Hence, glucose alone did not modify RBC velocity in normal tissue. Following galactose injection, the velocity decreased by 56% at 10 min. The velocity remained constant until 70 min and then slightly decreased at this time. The galactose values significantly differed from the control values between 10 and 40 min (Fig. 3, middle).

The results obtained from flow rate measurements are shown in Table 1 and Fig. 3, right. Following saline injection, the flow rate significantly decreased by 60% at 60 min and reached a value 87% below the base line at 110 min. Again, similar results were observed following glucose injection. The flow decreased by 44% at 60 min and a 52% decrease was observed at 110 min. Similar to the RBC velocity results, there was no significant difference between the normalized control and glucose values. Following galactose injection, a significant decrease of 53% was observed at 10 min. The flow then remained constant until 70 min and then decreased, reaching a value 71% below the base-line value at 110 min. The galactose values significantly differed from the control values between 10 and 40 min.

Neoplastic Tissue. The average results obtained on diameter, RBC velocity, and flow rate for neoplastic tissue are shown in Table 2 (N = 10 vessels). The normalized values are shown in Fig. 4. Again, statistical analysis was performed, according to the same two methods used in the normal tissue study, to compare each value with its base-line value and to compare the control values with the glucose/galactose values.

There was no significant change in vessel diameter in the tumor following saline, glucose, or galactose injection (see Fig. 4, left, and Table 2). The average diameters used in these studies were 43.51 ± 1.11, 32.15 ± 0.81, and 40.18 ± 0.34 for the control, glucose, and galactose experiments, respectively. Following saline injection, the velocity increased by 15% at 10 min and then returned to base line. At 70 min, the velocity then decreased by 30% and continued to decrease reaching a value

Fig. 2. Blood glucose (mg/100 ml) versus time after glucose (6 g/kg i.v.) and saline (8 ml/kg i.v.) injection. (N = 7 rabbits, control; N = 11 rabbits, glucose).

Points, mean; bars, SE; *, P < 0.05.

Table 1 Microcirculatory parameters in mature granulation tissue

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Diameter (μm)</th>
<th>RBC velocity (mm/s)</th>
<th>Flow rate (pl/s)</th>
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<tr>
<td></td>
<td>Control</td>
<td>Glucose</td>
<td>Galactose</td>
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<tr>
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<td>35 ± 4</td>
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* Mean ± SE.

* P < 0.05.
Fig. 3. Diameter (left), RBC velocity (middle), and blood flow rate (right) in normal tissue were normalized with respect to the base-line values and plotted as a function of time following the injection of saline (8 ml/kg i.v.; control; N = 15 vessels) and following the injections of glucose (6 g/kg i.v.; N = 15 vessels) and galactose (6 g/kg i.v.; N = 15 vessels). Unpaired t tests were performed to compare the results obtained following glucose/galactose injection with those obtained following saline injection. *, P < 0.05; points, mean; bars, SE.

Table 2. Microcirculatory parameters in neoplastic tissue

Table 2. Microcirculatory parameters in neoplastic tissue for control, glucose, and galactose studies (N = 10 vessels). A paired t test was performed to compare each value with its base-line value.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Diameter (μm)</th>
<th>RBC velocity (mm/s)</th>
<th>Flow rate (pl/s)</th>
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<tr>
<td></td>
<td>Control</td>
<td>Glucose</td>
<td>Galactose</td>
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<td>38 ± 4</td>
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<tr>
<td>110</td>
<td>42 ± 4</td>
<td>30 ± 4</td>
<td>43 ± 4</td>
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</table>

* Mean ± SE.

b P < 0.05.

Fig. 4. Diameter (left), RBC velocity (middle), and blood flow rate (right) in neoplastic tissue were normalized with respect to the base-line values and plotted as a function of time following the injection of saline (8 ml/kg i.v.; control; N = 10 vessels) and following the injections of glucose (6 g/kg i.v.; N = 10 vessels) and galactose (6 g/kg i.v.; N = 10 vessels). Unpaired t tests were performed to compare the results obtained following glucose/galactose injection with those obtained following saline injection. *, P < 0.05; points, mean; bars, SE.

71% below the base line at 110 min. Following glucose injection, the velocity decreased by 56% at 10 min and followed by a slight increase at 20 min, the velocity continued to decrease reaching 100% inhibition at 110 min. The normalized values significantly differed from the normalized control values from 10 to 80 min. Following galactose injection, the velocity decreased by 36% at 30 min and continued to decrease, also reaching 100% inhibition at 110 min. The normalized values significantly differed from the normalized control values from 30 to 70 min (Fig. 4, middle).

The results obtained from the flow rate measurements are shown in Table 2 and Fig. 4, right, and are similar to the RBC velocity results. Following saline injection, the flow remained constant until 80 min. At this time, it decreased by 79%. Following glucose injection, the flow decreased by 49% at 10 min and continued to decrease, reaching 100% inhibition at 110 min. Following galactose injection, the flow decreased by 37% at 30 min and continued to decrease reaching 100% inhibition at 110 min. The normalized values for glucose and galactose significantly differed from the normalized control values from 10 to 70 and 30 to 70 min, respectively.

WBC Adhesion

Before choosing a vessel for WBC adhesion studies, a series of experiments were performed to observe adhesion in arterioles, venules, and capillaries in both normal and tumor tissue.
In both arterioles and capillaries, WBC adhesion was minimum. At the most, one or two cells adhered during the 5-min period and this was not altered following glucose or galactose injection. Most of the WBC adhesion was observed in the venules. Therefore, we chose to concentrate on venules for our quantitative analysis.

The average results obtained on WBC adhesion are shown in Table 3 for normal (N = 6 vessels; glucose; N = 9 vessels, galactose) and tumor tissue (N = 7 vessels; glucose; N = 6 vessels, galactose). A paired t test was performed to determine if there was a significant difference between each value and its base-line value. (In Table 3, Footnote b indicates P < 0.05.). In normal tissue, modifications in WBC adhesion following glucose and galactose injection were not consistent. This is evident by the paired t test which showed no difference between the values and their base-line values. The average diameters of the vessels studied in normal tissue were 38.35 ± 7.13 and 36.16 ± 3.25 μm for glucose and galactose injections, respectively.

In tumor vessels, immediately following glucose and galactose injection, the modification in adhesion was not consistent. Some vessels showed an increase whereas others showed a decrease. Between 40 and 45 min, the adhesion decreased by 70% following glucose injection. Between 30 and 35 min, the adhesion decreased by 73% following galactose injection. The average diameters of the vessels studied in tumor tissue were 35.83 ± 4.15 and 35.33 ± 3.94 for glucose and galactose injections, respectively. There was no correlation between WBC adhesion and vessel diameter in either the normal or tumor vessels.

Systemic Blood Viscosity

The average results obtained from whole blood viscosity measurements following glucose and galactose (N = 6 rabbits) injections are shown in Table 4. A paired t test was performed to determine if there was a significant difference between the values and their base-line values (In Table 4, Footnote b indicates P < 0.05.).

Following glucose injection, the viscosity increased by approximately 15% at high shear rates of 45.0–225.0 s⁻¹ 75 min following glucose injection. There was no significant change in viscosity following galactose injection. Results also showed that hematocrit did not significantly change following glucose or galactose injection.

**Table 4 Average systemic blood viscosity (cp) following glucose and galactose injections (6 g/kg, i.v.)**

Viscosity was measured using a Wells-Brookfield cone/plate viscometer. A paired t test was performed to compare each value with its base-line value.

<table>
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<th>Shear rate (s⁻¹)</th>
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* Mean ± SE.  
* P < 0.05 (N = 6 rabbits).

Discussion

The main objective of this study was to determine the response of the microvasculature to glucose and galactose injection. Also, as reviewed in Ref. 16, the effects of anesthesia on normal and tumor tissue blood flow are not well defined. The magnitude and type of response depend not only on the anesthetic agent used but also on the dose and type of tissue studied. In this investigation, we proved that the doses of Nembutal used in these studies definitely affect the blood flow in the system used. Hence, these modifications must be taken into account when interpreting blood flow response. Our results are in agreement with those obtained by Kallman et al. (18) and Johnson et al. (19) who observed a reduction in tumor blood flow in various sarcomas following Nembutal injection.

The results obtained on microvascular response following glucose injection showed that hyperglycemia produced no change in lumen diameter, RBC velocity, or flow rate in the normal tissue over and above those due to anesthesia. However, in the neoplastic tissue, glucose decreased the RBC velocity and flow rate by approximately 50% 5 min after injection. The flow and velocity continued to decrease reaching 90% inhibition 65 min following injection.

Our microcirculatory flow measurements in VX2 carcinoma are in agreement with the observations of Algire and Legallais (1) who studied the response of L sarcoma, Sarcoma 37, and C3HBA mammary adenocarcinoma grown in dorsal chambers in mice. Following i.p. injection of 1 ml of a 20% glucose solution, they observed flow stasis 180 min postinjection in all tumor lines. Our results are also in agreement with those of Calderwood and Dickson (6) who observed a 90 and 98% decrease 1 and 2 h postinjection in Yoshida sarcoma tumor implanted in rats following glucose injection (6 g/kg, i.p.), and also with the results of Jain et al. obtained in Walker 256 carcinoma in rats using the microsphere technique (10) or the temperature drop (9). No one prior to this work reported data on vessel diameters.

The results obtained on the microvascular response following galactose injection showed that galactose decreased the flow in neoplastic tissue by 37% 25 min following injection and the flow continued to decrease to 90% below the base line 65 min following injection. Galactose produced similar modifications in RBC velocity. Vessel wall diameter did not change significantly following galactose injection. Unlike glucose, galactose also produced a decrease in blood flow in normal tissue. The flow decreased by 55% 5 min after injection. It then remained...
constant until 55 min after injection, and at this time anesthesia produced a further decrease.

To the best of our knowledge, this is the first investigation performed to quantify blood flow modification in both normal and tumor tissue at the microvascular level following galactose injection. The results obtained in the tumor are in agreement with those obtained in the macroscopic study performed by Dickson and Calderwood (20) who observed a 95% decrease in the blood flow of Yoshida Sarcoma 60 min following injection. These results also support the work of Jain et al. (9), who observed a temperature drop of approximately 5°C in both normal s.c. tissue and W256 tumors in rats 2 h following injection of galactose (6 g/kg i.p.).

Mechanisms of Blood Flow Reduction. We have shown recently that there is a 25% reduction and redistribution of cardiac output following hyperglycemia which can account for part of the blood flow reduction in tumors (10). Similarly, we have found that galactose also decreases cardiac output by 30%.

The local modifications in the microcirculation must account for the rest of blood flow reduction. Local blood flow in a tissue is a function of the pressure difference between the arterial and venous sides and also the flow resistance which depends on the apparent viscosity of the blood and the geometrical resistance. This is demonstrated by the relationship

\[ Q = \frac{f_i(D)f_a(L)f_v(\mu)f_p(\Delta P)}{ XM/XM'/XAP} \]  

(B)

which is based on the Hagen-Poiseuille equation for parabolic flow in a tube. Hence, if mechanisms responsible for flow modification are to be determined it is important to study (a) vascular morphology (i.e., diameter, D, and length, L, of the vessels), (b) blood rheology (i.e., viscosity, \( \mu \)), and (c) microvascular pressure, \( \Delta P \). In what follows, we will discuss the modifications of these parameters following glucose and galactose injection and propose a theoretical mechanism based on the experimental evidence.

The pressure drop across a vasculature is equal to the pressure difference between its arterial and venous sides. The pressure on the venous side is nearly zero (21). We have found (10) that the mean arterial pressure and heart rate do not change for up to 1 h following glucose injection in unanesthetized rats (6 g/kg i.v.). If we assume that the rabbit’s response to glucose is similar to that of rats, we may assume that changes in \( \Delta P \) are negligible. In similar experiments, we found that when injecting galactose, mean arterial pressure decreases by 25% during the injection and returns to the base-line value within 30 min after injection depending upon the rate of injection. Therefore, unlike glucose, we cannot eliminate \( \Delta P \) as a possible mechanism.

Tissue blood flow may also be modified in response to a pharmacological agent due to constriction or dilation of blood vessels. In addition, adhesion of WBC may decrease the “functional” diameter of the vessels. As shown in Figs. 3, left, and 4, left, the diameter of blood vessels did not change following glucose/galactose injections. Also, both qualitative and quantitative analyses showed no increase in WBC adhesion in normal or tumor tissue due to the agents (Table 3). Instead, a 70% reduction in adhesion was observed in the tumor vessels 40 min postinjection. This reduction was presumably due to a decrease in WBC flux caused by the blood flow reduction. No significant change in adhesion was observed in the normal tissue.

For a fixed shear rate and temperature, the viscosity of blood (\( \mu \)) is determined by the concentration of plasma proteins and the rigidity and number of RBC. Both local and systemic viscosity of blood may increase due to increased concentration of solutes dissolved in the blood (e.g., glucose, galactose, proteins). Separate studies have shown that the microvascular permeability of normal or tumor vessels remains constant following glucose or galactose injections (22). Also, results obtained from our systemic blood viscosity measurements showed that glucose produced a slight increase (15%) in systemic viscosity at high shear rates. This decrease did not occur, however, until 80 min postinjection. Galactose did not significantly affect blood viscosity (Table 4).

Local viscosity may increase due to increase in RBC rigidity. Traykov and Jain (23) have shown that glucose itself increases the rigidity of RBC as measured by a micropipet aspiration method. In addition, Crandall et al. (24) have shown low pH also increases the rigidity of RBC although at a slower rate than glucose. As stated in the “Introduction,” injection of glucose lowers tumor pH by two pathways: direct conversion of glucose into lactic acid; and slower egress of lactic acid from tumors due to reduced blood flow rate (9). As RBC become rigid, they do not migrate to the center of vessels to the extent to which deformable cells do (25). As a result, the thickness of the “plasma skimming” layer is reduced, leading to an increase in local hematocrit. This increase in local hematocrit would then increase local viscosity in the microvessels (25). Further, Chadwick (26) has shown theoretically that tortuous vessels offer higher resistance to blood flow to begin with. Finally, as shown by Simchon et al. (27), the rigid cells may be trapped in the tortuous vasculature of neoplastic tissue leading to further reduction of tumor blood flow. Results have shown that galactose neither decreases pH in a tumor (9) nor increases RBC rigidity significantly (23).

Based on the experimental observations of other investigators and our own findings, we postulate that the governing mechanisms responsible for tumor blood flow reduction during hyperglycemia include cardiac output reduction and increase in local viscosity due to increase in RBC rigidity. Our proposed framework is shown in Fig. 5. This increase in local viscosity due to hyperglycemia has been verified recently by Sevick and Jain using an isolated tumor preparation. It should be noted that microvascular pressure modification cannot be completely excluded as a possible mechanism. It is possible that the pressure drop across a vessel can be modified during hyperglycemia due to osmotic imbalances and hyperglycemic shock. A definite need exists to measure this pressure directly, to understand its role.

Fig. 5. Various systemic and local mechanisms thought to be responsible for flow reduction in tumors during hyperglycemia.

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E. Sevick and R. Jain, unpublished results.
Experimental observations have shown that the mechanisms responsible for blood flow reduction in both normal and tumor tissues following galactose injection also include systemic and local effects. Based on our results and those reported in the literature, systemic effects include reduction in systemic pressure and cardiac output immediately following galactose injection. Unlike glucose, the local mechanisms for galactose are not completely understood. They may include changes in microvascular pressure (caused by osmotic imbalance and hyper-galactosemia shock) and microvascular blood viscosity (caused by plasma volume change resulting from osmotic imbalance). Also, while Traykov and Jain (23) showed no modification in the rigidity of RBC exposed to isotonic galactose solution, Chien et al. (25) have shown that osmotic imbalances can affect RBC rigidity. Hence, RBC rigidity occurring in vivo cannot be eliminated as a possible mechanism. A definite need exists to measure microvascular pressure and viscosity in individual blood vessels to quantify the mechanisms of flow reduction due to galactose.

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Effect of Glucose and Galactose on Microcirculatory Flow in Normal and Neoplastic Tissues in Rabbits

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