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Effect of Hyperglycemia on Blood Flow, pH, and Response to Hyperthermia (42°) of the Yoshida Sarcoma in the Rat[1]

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ABSTRACT

Hyperglycemia (blood glucose, >20 mmol/liter) caused a 90 to 100% inhibition of blood flow in the solid Yoshida sarcoma of rat feet, as measured by the fractional distribution of ^{86}Rb and ^{133}Xe clearance. Blood flow through the normal gastrocnemius muscle was increased by 50%, while liver blood flow remained unaltered. Hyperglycemia abrogated the temperature differential (approximately 1°) between the heating bath and the tumor, promoting more uniform tumor heating.

During the period of reduced blood flow, the pH of the tumor extracellular fluid, measured by miniature glass electrode, declined from 7.19 to 6.63 due to decreased efflux of lactate from the tumor. Tumor intracellular pH, measured by partitioning of dimethyloxazolidinedione across the cell membrane, increased from 7.21 to 7.36.

At a very high blood glucose concentration (50 mmol/liter), the tumor was isolated from the host, with almost total blockade of water, chloride, glucose, lactate, and dimethyloxazolidinedione exchange between the tumor and the blood.

Hyperglycemia therefore represents a convenient means of isolating the Yoshida sarcoma from the host blood supply to enable more selective treatment with hyperthermia and possibly other modalities.

INTRODUCTION

Temperatures of 41–43° selectively destroy many types of malignant cells (21, 24). Above 43°, there is increasing damage to normal tissues, and the use of heat to treat tumors resistant to 41–43° (5, 7) depends on selectively heating the tumor or the use of a potentiator of the hyperthermia. Von Ardenne (26) suggested the use of glucose as such a sensitizer. Hyperglycemia was envisaged as inducing lactic acidosis in tumors by exploiting the increased glycolysis associated with malignant cells (29). A decrease in tumor pH to approximately 6.5 would, according to Von Ardenne's hypothesis, labilize lysosomal membranes; at this decreased pH, heating at 42° would lead to tumor autolysis (26). The present report describes the effect of hyperglycemia on extra- and intracellular pH in the solid Yoshida rat sarcoma and presents evidence that hyperglycemia acts by selectively inhibiting tumor blood flow, thus facilitating more uniform tumor heating.

MATERIALS AND METHODS

Tumor System. Details of the history, maintenance, and growth characteristics of the Yoshida sarcoma are described elsewhere (8). For this work, the tumor was grown in the dorsum of the left hind foot of the rats to a volume of 1.0 to 1.5 ml (8 to 10 days after implantation).

Radioisotopes. $^{3}H_2O$ (specific activity, 5 Ci/ml), $Na^{36}Cl$ (chlorine, 3 mCi/g), $^{86}RbCl$ (rubidium, 2 to 10 Ci/g), ^{133}Xe dissolved in 0.9% NaCl solution (xenon, 2 to 10 Ci/ml), 2-[^{3}H]deoxyglucose (15 Ci/mol), and L-[*U*-^{14}C]lactic acid, sodium salt (5 to 20 Ci/mol), were obtained from The Radiochemical Centre, Amersham, England. 5,5-Dimethyloxazolidine[2-^{14}C]-2,4-dione (DMO,[2] 2 to 10 Ci/mol) was obtained from NEN Chemicals GmbH, Dreichenhain, West Germany.

Glucose Determination. Blood glucose was measured by glucose oxidase using a blood sugar test combination [Boehringer Corporation (London) Ltd., Bell Lane, Lewes, E. Sussex, England]. The blood glucose estimation was carried out on 0.1 ml of deproteinized heart blood (0.1 ml of whole blood in 1.0 ml of 0.16% uranyl acetate). For tumor glucose determination, 0.3 to 0.5 g tumor tissue was homogenized in 2.0 ml distilled water using a Polytron microhomogenizer (Northern Media Supply Ltd., Hull, England) at full speed for 1 min. To 0.5 ml of this homogenate was added 1.0 ml of 0.16% uranyl acetate for deproteinization. The solution was vigorously mixed and centrifuged at 3000 rpm for 10 min, and the glucose was estimated in 0.1 ml of supernatant. Results were expressed as mmol glucose per liter blood or tumor.

Tumor pH Measurement. For determination of intracellular pH (pH_i), rats were given an i.p. injection containing 50 μCi $^{3}H_2O$, 1 μCi ^{36}Cl, and 1 μCi [^{14}C]DMO in 1.0 ml 0.9% NaCl solution. Following sacrifice of animals 2 hr after isotope injection, tissue concentrations of radiochemicals and pH_i were determined as described previously (3).

Tumor extracellular pH (pH_e) was measured by miniature capillary glass electrodes (type MI 400) with a 1-mm-diameter tip and a reference microelectrode (type MI 401) filled with 3 M KCl saturated with AgCl (Microelectrodes, Inc., Londonderry, N. H.). The electrodes were coupled by high-impedance amplifier to a digital pH meter (type PHM 63; Radiometer, Copenhagen, Denmark). For anesthesia, the rats were given 0.1 ml of a 1:5 dilution of Nembutal veterinary i.p. (pentobarbitone sodium, 60 mg per ml; Abbott Laboratories, Queenborough, Kent, England) per 50 g of body weight. Narcosis was maintained by additional small doses of the barbiturate as required. The anesthetized rat was immobilized on an electrically insulated board, the distal 1 cm of its tail was amputated, and the bleeding tail was immersed in a 250-ml Erlenmeyer flask containing 200 ml 0.9% NaCl solution and the reference microelectrode. For measurement of tumor pH, the glass microelectrode was inserted to a depth of 3 to 5 mm through a small incision in the upper surface of the tumor. The electrode was

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[2] The abbreviations used are: DMO, 5,5-dimethyl-2,4-oxazolidinedione; pH_i, intracellular pH; pH_e, extracellular pH; i.t., intratumoral.

then secured vertically in position and connected to the digital pH meter, and the system was left to stabilize. When electrode stability was achieved, a pH reading with a variation of $<\pm 0.03$ unit/hr was recorded; this usually required 40 to 60 min. In experiments on hyperglycemia, an i.p. injection of 50% glucose [6 g/kg body weight (11)] was given at this point, or an i.v. infusion of 20% glucose was started via the femoral vein and pH was monitored for 4 to 9 hr. At the end of the experiment, the animal was sacrificed, and the capillary electrode track was examined to ensure that it had been situated in viable tumor.

Blood Flow Measurement. Blood flow was determined by 2 methods, the fractional distribution of ^{86}Rb (22) and ^{133}Xe clearance (2). In the first method, animals were given injections of 100 μCi of ^{86}Rb in 0.1 ml of 0.9% NaCl solution into the right femoral vein. This isotope becomes distributed between tissues in a concentration proportional to the tissue fraction of the cardiac output (22). The rats were sacrificed 40 sec after injection, and blood flow values were calculated by multiplying the cardiac output fraction for the organ by the total cardiac output for a pentobarbitone-anesthetized 200-g rat (14).

Xenon clearance was measured after i.t. injection of 50 μCi of ^{133}Xe in 0.05 ml of 0.9% NaCl solution. Tumor radioactivity was detected using a potassium iodide scintillation crystal coupled to a ratemeter and chart recorder. The crystal was positioned 1 to 2 cm above the tumor and shielded to avoid detection of ^{133}Xe in the lungs of the animal. Radioactive decay followed a multiexponential function (Chart 4), and blood flow was calculated from the half-time of tumor clearance ($t_{1/2}$) using the equation:

$$\text{Blood flow (ml/min)} = \frac{\log_e 2 \times \lambda}{t_{1/2}\text{ (min)}} \qquad (2)$$

where λ is the partition coefficient for ^{133}Xe between tumor cells and blood.

Tumor Angiography. The vascular network of 2- to 3-ml Yoshida sarcomas growing in the leg muscles of the rats was demonstrated by X-ray photography after injection of contrast medium at laparotomy into the aorta 1 cm above the iliac bifurcation. Leg tumors were used because little success was achieved in demonstrating the arterial system distal to the ankle by this technique. Photographs were taken 18 sec after injection of 8 to 10 ml 45% Hypaque (sodium diatriozate; Winthrop Laboratories, Surbiton. Surrey, England) through a 21-gauge needle.

Tumor Hyperthermia. Tumors were heated by water bath immersion. Bath and tumor temperatures were simultaneously monitored by thermistor probes as described in an earlier publication (8).

RESULTS

Effect of Hyperglycemia on Tumor pH. The effect of glucose administration by i.p. injection or i.v. infusion on blood and tumor glucose concentrations is shown in Chart 1. The i.p. injection of glucose (6 g/kg) caused a rapid increase in blood glucose level from a mean of 2.8 to a level greater than 30 mmol/liter by 30 min. A level in excess of 20 mmol/liter was maintained for 4 hr after injection, decreasing to control values by 6 hr. When a higher glucose dose was given by infusion (Chart 1; total glucose dose over 8 hr, 16 g/kg), the blood glucose concentration increased rapidly to 33 mmol/liter at 30 min and continued to increase to a plateau concentration of approximately 60 mmol/liter by 4 hr. Tumor glucose concentration following both glucose dose schedules showed a temporary increase, declining again to trace levels by 4 hr, while the blood glucose level remained elevated (Chart 1).

The effect of both glucose regimens on tumor pH is shown in Table 1. Following i.p. glucose injection, tumor pH_e, measured by capillary electrode, decreased from 7.19 to a minimum of 6.63 within 4 hr; pH_i, however, showed a slight but not significant ($p > 0.05$) pH increase from 7.21 to 7.36. Increasing the blood glucose level by infusion did not produce any further decrease in pH_e compared to the single i.p. injection, pH_e decreasing from 7.19 to a minimum of 6.70 in 4 hr; pH_i could not be measured under infusion conditions because ^3H_2O, [^{14}C]DMO, and ^{36}Cl did not enter the tumor (Chart 2).

Effect of Hyperglycemia on ^3H_2O, ^{36}Cl, and [^{14}C]DMO Exchange between the Tumor and Host. The concentration of ^3H_2O, [^{14}C]DMO, and ^{36}Cl in tumors at the fourth hr of glucose infusion was 1 to 2% of that in controls (Chart 2*a*). No inhibition of isotope uptake into the normal tissues was observed under these conditions. There was also inhibition of isotope exchange between the tumor and the host; this prevented isotopes injected into the tumor from entering the plasma and equilibrating with the normal tissues (Chart 2*b*). Isotopes injected into the tumor at 2 hr after infusion began were present in the host tissues at 4 hr in concentrations of less than 1% of the tumor level. Under normal conditions, isotopes injected into the tumor equilibrated freely with the host tissues in 2 hr (Chart 2*c*). There was no inhibition of isotope uptake into the Yoshida sarcoma at the lower level of hyperglycemia induced by a single glucose injection at 6 g/kg.

Tumor Blood Flow during Hyperglycemia. Uptake of ^{86}Rb

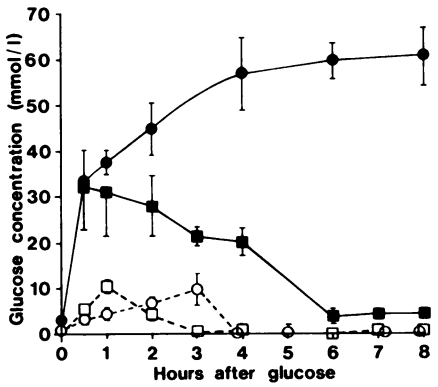

Chart 1. Blood (■) and tumor (□) glucose concentrations after injections of glucose (6 g/kg rat i.p.). Another series of animals was infused with 20% glucose at 2.4 ml/hr and also given 0.75-ml i.p. injections of 50% glucose at 0, 0.5, 1, and 1.5 hr. ●, blood glucose levels; ○, tumor glucose levels. *Points*, means of 5 to 7 determinations (animals); *bars*, S.D.

Table 1

Effect of hyperglycemia on pH_e and pH_i of Yoshida sarcoma

Condition	Tumor pH		
	pH_e (electrode)		pH_i (DMO)
Untreated control	7.19 ± 0.13[a]	(20)[b]	7.21 ± 0.16 (48)
4 hr after i.p. glucose injection	6.63 ± 0.21	(17)	7.36 ± 0.14 (12)
4 hr after glucose infusion	6.70 ± 0.10	(8)	

[a] Mean ± S.D.
[b] Numbers in parentheses, number of tumors (animals) investigated.

by Yoshida sarcoma slices was measured *in vitro* after hyperglycemia *in vivo* (Table 2). Differences in uptake of the isotope between slices from 6 hyperglycemic animals and 6 control (normoglycemic) animals were not significant even at the 10% level. It was concluded that hyperglycemia has no marked effect on uptake of ^{86}Rb by Yoshida sarcoma cells and should not interfere with the measurement of blood flow by altering ^{86}Rb transport into the cells.

Following glucose infusion, blood flow measured by ^{86}Rb uptake decreased progressively from 0.41 mg per g, dry weight, per hr in control tumors to 0.06 ml per g per hr at 1 hr, to 0.03 ml per g per hr at 2 hr, and to trace levels by 4 hr (Chart 3). Tumor blood flow was inhibited for as long as the blood sugar level remained elevated. A single i.p. injection of glucose (6 g/kg) also caused a marked decrease in tumor blood flow from 0.41 to 0.04 ml/g/hr at 1 hr and a minimum of 0.01 ml/g/hr at 2 hr. Tumor blood flow remained at a decreased level of 0.03 ml/g/hr at 4 hr, gradually increasing to 50% of control level by 8 hr. The decrease in tumor blood flow at high blood glucose levels was not dependent on tumor site but also occurred in 1.0- to 1.5-ml tumors growing s.c. in the flank and in 1.0- to 3.0-ml i.m. tumors in the rat legs.

In the gastrocnemius, blood flow increased by 50% in the presence of hyperglycemia (20 or 50 mmol/liter), while blood flow through the liver was not significantly altered at these levels of blood glucose.

The finding of inhibition of blood flow in the Yoshida sarcoma by hyperglycemia was confirmed using the ^{133}Xe clearance technique to measure blood flow. Xenon clearance curves recorded 4 hr after commencement of the 2 glucose regimens are shown in Chart 4. After infusion or injection of glucose, the degree of blood flow inhibition, as indicated by ^{133}Xe clearance, was essentially similar to the values obtained by ^{86}Rb uptake (Chart 3).

Impairment of the tumor blood supply in hyperglycemic hosts was also indicated by angiography (Fig. 1). In hyperglycemic animals, the number of demonstrable tumor blood vessels was greatly reduced compared to controls, and the patency of vessels supplying the normal tissues is evident.

Exchange of Labeled Metabolites between the Tumor and Host during Hyperglycemia. Chart 5 illustrates further the differential effect of the 2 levels of hyperglycemia (achieved by glucose injection or infusion) on exchange of metabolites between tumor and host. At a blood glucose level of 20 mmol/liter, there was an 85% inhibition of 2-[^{3}H]deoxyglucose uptake into the tumor. Glucose infusion (blood glucose, 50 mmol/liter) led to 100% inhibition of 2-deoxyglucose uptake into the tumor.

A similar pattern was found for lactate egress from the tumor (Table 3). When [^{14}C]lactate was injected directly into the Yoshida sarcoma in normoglycemic hosts and when the rats

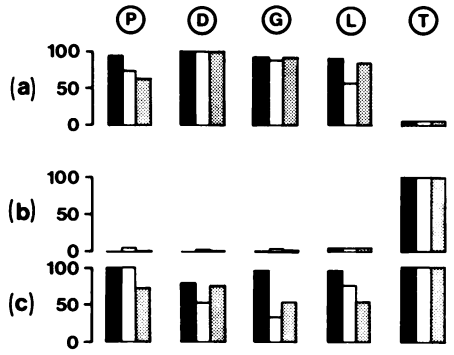

Chart 2. Effect of hyperglycemia on uptake of $^{3}H_2O$ (■), ^{36}Cl (□), and [^{14}C]-DMO (▨) by rat tissues. In *a*, glucose was given by i.v. infusion, and at 2 hr the animal was given an i.p. injection of the isotope mixture (50 μCi $^{3}H_2O$, 1 μCi ^{36}Cl, and 1 μCi [^{14}C]DMO in 1.0 ml of 0.9% NaCl solution). Infusion was continued for a further 2 hr; then the animal was sacrificed, and isotope concentrations (cpm/g tissue, wet weight) were determined as percentage of cpm compared to normoglycemic controls. *P*, plasma; *D*, diaphragm; *G*, gastrocnemius; *L*, liver; *T*, Yoshida tumor. In *b*, after 2 hr glucose infusion, the isotope mixture (5 μCi $^{3}H_2O$, 0.1 μCi ^{36}Cl, and 0.1 μCi [^{14}C]DMO in 0.1 ml 0.9% NaCl solution) was injected into the tumor, and infusion was continued. Results are expressed as percentage of cpm (cpm/g tissue) compared to activity in the tumor 2 hr after isotope injection. Controls for *b* were normoglycemic animals sacrificed 2 hr after i.t. isotope injection. In *c*, results are again cpm/g tissue expressed as a percentage of cpm remaining in the tumor. Results are means from 5 experimental and 5 control animals in each group. S.D.'s were 5 to 10% of mean value in each case.

Table 2

Effect of hyperglycemia on ^{86}Rb uptake by Yoshida sarcoma slices

Tumors were from 6 normoglycemic animals after 4 hr sagittal anesthesia and 6 hyperglycemic animals anesthetized for 4 hr after an injection of glucose (6 g/kg i.p.). Immediately upon removal, thin tumor sections (less than 1 cu mm) were prepared (9). The slices were washed in 5 ml of Waymouth's medium, containing 0.1% albumin, and 100-mg aliquots were weighed into 5-cm Petri dishes.

To the tumor slices were added 3 ml of medium containing ^{86}Rb (2.5 μCi/ml), and the slices were incubated at 37° for 2 hr (16). The time elapsing between removal of tumor from the animal and addition of ^{86}Rb to culture medium was less than 45 min. After equilibration (2 hr), slices were washed 3 times in 5 ml of ice-cold phosphate-buffered saline (pH 7.4) (9). Radioactivity was expressed as cpm/g dry weight. ^{86}Rb incorporation in slices from hyperglycemic animals was compared to incorporation in control slices using a paired *t* test (28).

Condition	^{86}Rb incorporation (cpm/g dry wt)	% of control	*t*	*P*
Normoglycemic	12,375,050			
Hyperglycemic	10,633,685	85.93	0.677	>0.10

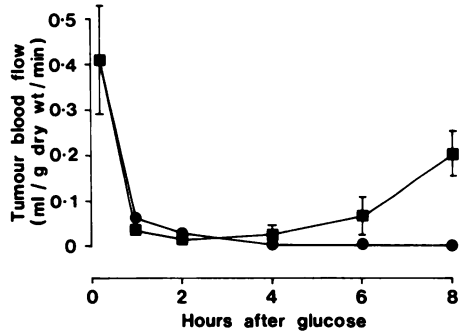

Chart 3. Effect of hyperglycemia induced by a single i.p. injection (■) or by i.v. infusion (●) on Yoshida sarcoma blood flow measured by ^{86}Rb uptake. *Points*, means of 5 determinations (animals); *bars*, S.D.

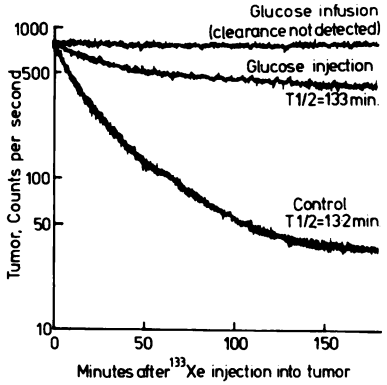

Chart 4. Clearance of ^{133}Xe after i.t. injection into the Yoshida foot sarcoma, showing sample chart recordings of ^{133}Xe clearance of individual tumors 4 hr after glucose injection, after 4 hr glucose infusion, and in a control (normoglycemic) animal.

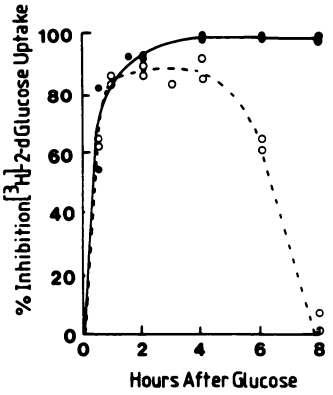

Chart 5. Inhibition of 2-[^{3}H]deoxyglucose ([^{3}H]-2-dGlucose) uptake in the Yoshida foot tumor after a single i.p. injection of glucose [6 g/kg; blood glucose, 20 mmol/liter for 4 hr (– – –)] or after i.v. glucose infusion [blood glucose, 50 mmol/liter (——)]. Each rat received 10 μCi isotope i.p. in 1 ml 0.9% NaCl solution; each *point* represents tumor activity in an animal sacrificed 30 min after injection. The analog 2-deoxyglucose has transport properties similar to those of glucose but is only slowly metabolized, making it a suitable marker for the study of glucose transport (10).

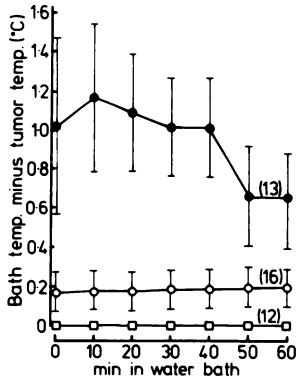

Chart 6. Temperature gradient between Yoshida sarcoma and water bath (at 43°) in control, normoglycemic animals (●), after a single i.p. glucose injection at 6 g/kg (○), or after i.v. glucose infusion (□). *Points*, means; *bars*, S.D.; *numbers in parentheses*, number of animals in each group.

Table 3

Effect of glucose (6 g/kg) on [^{14}C]lactate efflux from tumors

[^{14}C]Lactate (1 μCi in 0.1 ml of 0.9% NaCl solution) was injected into tumors 2 hr after i.p. administration of glucose at a dose of 6 g/kg. Animals were sacrificed 30 min after the lactate injection, and ^{14}C activity was determined in the tumor and normal organs. In controls, tissue radioactivity was determined 30 min after an i.t. injection of [^{14}C]lactate. The ^{14}C activity in the tissues is presented both as cpm/g, wet weight, and as percentage of tumor radioactivity, the tumor value being taken as 100%.

	Control		Hyperglycemic	
Tissue	cpm/g	%	cpm/g	%
Tumor	101,219 ± 25,196[a]	100	365,980 ± 52,165	100
Plasma	24,332 ± 1,655	24.0	18,652 ± 4,858	5.1
Liver	6,319 ± 1,965	6.2	2,198 ± 796	0.6
Diaphragm	25,126 ± 5,316	24.8	10,950 ± 4,965	3.0
Gastrocnemius	18,926 ± 3,168	18.7	7,916 ± 1,615	2.16

[a] Mean ± S.D. of 4 determinations (animals).

were sacrificed 30 min later, the plasma and other organs contained 43% of the total radioactivity of the carcass. After glucose loading of the host at 20 mmol/liter for 2 hr, only 9.8% of the activity injected into the sarcoma was recovered in tissues apart from the tumor (illustrated for sample organs in Table 3). After 4 hr infusion (blood glucose 50 mmol/liter), almost 100% of the cpm remained in the tumor at 30 min after isotope injection.

Effect of Hyperglycemia on the Thermal Load Required for Tumor Heating at 42°. The decreased tumor blood flow caused by hyperglycemia diminished the thermal load necessary for tumor heating and also promoted a more uniform tumor temperature during heating (Chart 6). In normoglycemic rats, the temperature gradient between the water bath at 43° and the tumor was approximately 1.0° (*i.e.*, tumor 42°) for the first 40 min of heating. This gradient decreased to 0.7° as the heat treatment was continued to 50 and 60 min. In tumors heated 4 hr after glucose injection at a dose of 6 g/kg, the temperature gradient was reduced to a mean of approximately 0.2° and remained at this level throughout the 60-min heating period. Following glucose infusion for 4 hr, the temperature gradient between tumor and bath was abolished (Chart 6).

The rectal temperature of hyperglycemic rats anesthetized by pentobarbitone was not significantly ($p > 0.05$) altered compared to that of control animals at a mean room air temperature of 25°. Normoglycemic animals had a rectal temperature of 34.2 ± 1.3° (S.D.) (8 rats), while animals after 4 hr glucose infusion had a temperature of 32.9 ± 1.7° (6 rats).

DISCUSSION

The concomitant changes in blood supply and pH_e in the Yoshida sarcoma after hyperglycemia suggest that the 3 effects are interrelated. The evidence for inhibition of blood flow in the tumor is strong; both tumor uptake and clearance of a wide range of chemical species was inhibited during hyperglycemia (Charts 2 and 5; Table 3), and quantitation of the inhibition by different methods (^{86}Rb distribution and ^{133}Xe clearance) yielded comparable results (Charts 3 and 4). The results confirm an earlier finding of Algire & Legallais (1) who found that hyperglycemia (blood sugar level unspecified) inhibited blood circulation in tumors growing in transparent chambers implanted in mice. In rat tumors, inhibition of blood flow during hyperglycemia has been reported more recently by Von Ardenne (27), who has proposed a hypothesis to account for the concomitant fall in tumor blood flow and pH_e (27). Hyperglycemia, it is postulated, leads to a stimulation of glycolysis with lactic acidosis in the tumor; erythrocytes entering the acidified tumor would be expected to undergo a pH-mediated change in membrane conformation causing a decreased flexibility (27). Low pH has been shown to alter erythrocyte membrane structure (25) and to increase blood viscosity [attributed to increased erythrocyte rigidity (17)]. Such erythrocytes, it is argued, would lack the flexibility needed to pass through narrow capillaries and would physically block the tumor vessels (27). The theory (27) therefore implicates a decrease in pH as the initiating event, with a resulting inhibition of tumor blood flow.

The present data do not support this hypothesis. Tumor blood flow decreased rapidly after glucose injection, and the curve of blood flow inhibition (Chart 3) was almost a mirror image of the blood glucose curve (Chart 1); blood flow decreased as blood glucose increased and flow increased as glucose decreased. Tumor pH_e declined progressively but much more slowly than blood flow, reaching a minimum in 3.5 to 4 hr [detailed in an earlier publication (6)]. Previous work also showed that glycolysis (both aerobic and anaerobic) in the Yoshida tumor was inhibited by 35 to 60% during hyperglycemia (6) rather than stimulated as predicted by Von Ardenne

(26). Within 30 min after glucose injection, however, there was a rapid accumulation of lactate in the tumor to 2 to 3 times the normal level (6). Again, the rapidity of this accumulation paralleled the elevation of blood sugar and the decrease in blood flow and was well in advance of the fall in tumor pH_e. The time sequence of events therefore favors inhibition of blood flow as the initial event, with a subsequent decrease in tumor pH_e secondary to arrest of lactate egress from the tumor. The maintenance of tumor pH_i at control levels despite a 0.6-pH unit fall in pH_e (Table 1) is probably due to intracellular buffering and active transport of protons out of the cell (20), as well as the self-limiting effect of hyperglycemia preventing access of glucose to the cells.

The report by Gullino *et al.* (15) of a considerable increase in glucose utilization by the Walker 256 carcinoma, hepatoma 5123, and fibrosarcoma 4956 rat tumors in the first few hr after hyperglycemia (blood glucose, ≥20 mmol/liter) would seem to be at variance with the present data. The utilization did reach a saturation level after 6 to 7 hr of hyperglycemia, although no values for tumor blood flow were quoted by the authors (15). Increased glucose utilization in tumors after hyperglycemia would imply that no rapid inhibition of tumor blood flow occurred. The discrepancy between the findings of Gullino *et al.* and the present study may be due to differences between experimental tumors used in the studies. Moreover, the tumors used in the investigations of Gullino *et al.* (15) were grown in ovarian tissue isolated from the other normal tissues and connected to the host blood supply by a single artery and vein. This is a considerably different situation to that of the tumors grown by simple s.c. or i.m. implantation as used in the present study. In these circumstances, the tumor blood supply is connected by an agglomeration of tumor-induced new vessels to the vascular beds of surrounding normal tissues (30). It is conceivable that blood flow in such tumors might be more susceptible to disruption than in tumors supplied by a single large artery and vein that constituted the original vasculature to a normal organ.

The effect of glucose on tumor pH would thus seem to be complex, depending on rates of glucose influx into the tumor, lactate production and efflux, and buffering power. The balance between these processes in a tumor and the effect of hyperglycemia upon pH_i/pH_e ratio may hinge on whether the glucose inhibits tumor blood flow and at what glucose concentration this inhibition occurs.

The mechanism for the selective decrease in tumor blood flow at high blood glucose level is not indicated by the current data. The selective nature of the effect of high blood glucose levels on tumor blood flow may be attributed to the known differences between the blood supply of normal and malignant tissues (14, 18). Tumor blood flow in general is more sluggish and less responsive to local and systemic control than is blood flow in normal tissues (14, 18). The normal and tumor microvascular systems also differ, the tumor vessels being composed of dilated, tortuous capillaries and sinusoids with a primitive, often discontinuous, wall (12, 30). Periods of stasis, followed by resumed blood flow, often in the contrary direction, are features of the tumor microcirculation (13). The major determinants of blood flow in tumor capillaries are the physical state of the blood and integrity of the microcirculation (19). While there is no evidence to suggest that hyperglycemia might change the radius of tumor blood vessels, any such alteration would have a drastic effect on blood flow, inasmuch as vessel resistance is inversely proportional to the fourth power of the radius (19). A more likely effect of hyperglycemia would be via increased blood viscosity. Blood flow is inversely proportional to viscosity (19), a property which, particularly at low shear rates, is largely due to erythrocyte aggregation (23). The tumor microvasculature, with a large resistance to blood flow, would tend to favor RBC aggregation (23). In these circumstances, the chemical forces acting between cells in the formation of aggregates become significant compared to the shearing forces of blood flow, with a resultant increase in viscosity (19, 23). Increase in blood viscosity has the potential, therefore, to initiate a vicious circle of further slowing of blood flow, more aggregates, and higher viscosity and thus upset the pre- to post-capillary resistance (23). The primary event precipitating blood flow inhibition when blood viscosity is rapidly increased by glucose may be some alteration in the erythrocyte membrane (adhesiveness, fluidity), platelet aggregation, or a change in the bulk properties of the blood (osmolality, hematocrit) or in the tumor vessels (constriction, blockage), after which the vicious circle described might be triggered. The process is reversible, inasmuch as tumor blood flow resumes when normoglycemic conditions are restored (Charts 1 and 3). Our concept, therefore, envisages an initial effect of hyperglycemia on tumor blood flow, and this may encompass afferent and efferent vessels and/or microvasculature, with subsequent effects on tumor pH. In Von Ardenne's hypothesis, the primary effect is a postulated decrease in pH within tumor capillaries, with subsequent interruption of tumor blood flow.

The glucose-induced specific inhibition of tumor blood flow, if a general finding, has broad potential for the investigation and treatment of tumors; use of the sugar in this context is rendered more attractive since it is a physiological substance. The low pH_e accompanying hyperglycemia may sensitize some tumors to hyperthermia as proposed by Von Ardenne (26, 27), although in the Yoshida sarcoma hyperglycemia caused no such thermal sensitization (6). In association with hyperglycemia, a reduced thermal load would suffice to heat the tumor (Chart 6), and the selective nature of the blood flow inhibition would permit normal tissues to be maintained at nondamaging temperatures by the cooling influence of the bloodstream. This would be of special importance in the treatment of malignant tissues not sensitive to heat damage at 42° (5, 7) and that currently require higher temperatures at which the differential sensitivity between normal and malignant tissues is lost (4). Blood flow inhibition by hyperglycemia would, in general, enable tumors to be treated or studied in isolation from the normal tissues, promoting increased specificity in cancer therapy. Examples of this application could be the deposition of high concentrations of drugs in glucose-isolated tumors by the techniques of interventional radiology and the ischemic infarction of tumors in a manner akin to embolization of the arterial supply.

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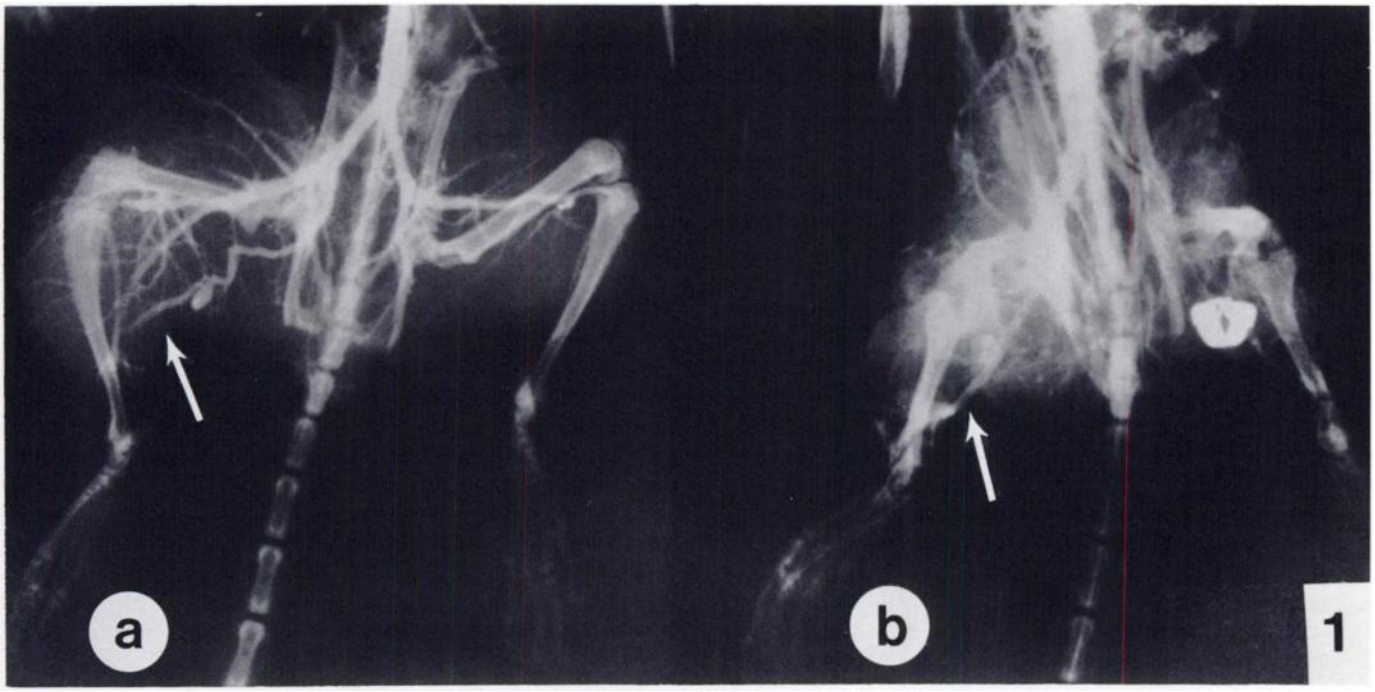

Fig. 1. Arterial blood supply in normal and Yoshida sarcoma-bearing (*arrow*) hind legs of untreated rat (*a*) and after 4 hr hyperglycemia [blood glucose, 52 mmol/liter (*b*)]. The network of vessels supplying the tumor is visualized in *a*, while only the main arterial trunk is clearly identified in *b*. Blood vessels were given injections of contrast medium (Hypaque) prior to X-ray photography. The metal clip used to seal the left femoral vein, used for the infusion, is seen in *b*. The different anatomical position of the pelvis in *a* and *b* resulted from uncontrollable reflex spasm of the animals' hindquarters due to the irritant nature of the contrast medium.