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Glucose absorption and utilization by rat embryos

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ABSTRACT There is little doubt that glucose plays a significant nutritional role in early somite embryos. The high glucose utilization of anaerobic glycolysis drops as the activity of the Kreb's cycle and terminal electron transport increase. Concurrently, maturation of mitochondrial cristae and dependence on oxygen supply are taking place. The neuroepithelium of the early somite rat embryo responds *in vitro* during culture by microvilliar lengthening when exposed to glucose levels of 50 mg/dl or more. At lower glucose concentrations both in whole embryo culture and inside the closed neural tube the microvilli are shorter. Lengthening of the microvilli at room temperature is produced only by d-glucose and 2-deoxyglucose, two hexoses that are absorbed and phosphorylated. Cytochalasin D which disrupts actin polymerization causes ballooning of the microvilli. A role of this microvillar elongation in degenerative changes seen in uncontrolled diabetes and on function of the immune system is proposed. The amniotic cavity is one major portal of entry for glucose during the early somite embryo stage. The 7-fold increase in volume of the amniotic cavity after day 10 allows the rat embryo to convert its axis from dorsal to ventral flexion.

KEY WORDS: *glucose metabolism, microvillus, glycolysis, actin polymerization, amnion, embryonic axis conversion*

Introduction

Glucose is the 'common currency' of metabolism (Lienhard *et al.*, 1992) and the developing embryo is relatively anaerobic which makes it even more dependent on glucose (Shepard *et al.*, 1970; Tanimura and Shepard, 1970; Neubert, 1973; Clough and Whittingham, 1983; Ellington, 1987a). In this review, we will attempt to summarize work on glucose metabolism from the Central Laboratory for Human Embryology accumulated during the past 25 years. This covers three areas: 1) the switch from anaerobic to aerobic glycolysis in early somite stage rat embryos (Tanimura and Shepard, 1970); 2) the role of the amniotic cavity in transfer of glucose (Park and Shepard, 1994); and 3) the control of glucose uptake by the microvillus of neural cells (Shepard *et al.*, 1993; Shepard and Park, 1994). Since each of these three studies was done at different periods with different techniques, abbreviated methods and results will be given separately, but the discussion of all three will be integrated at the end of the paper.

One of the main and indispensable methods used in these studies was whole embryo culture developed by Denis New (New, 1967; New *et al.*, 1973). These whole embryo culture methods have been widely used to study the mechanisms of teratology (Shepard *et al.*, 1976). Each year about twenty five abstracts from the Teratology Society meetings employ whole embryo culture as their primary technique.

Methods and Results

Glucose uptake and utilization by rat embryos: anaerobic to aerobic glycolysis

Sprague-Dawley rats were time-mated and obtained from Tyler Laboratories (Bellevue, WA, USA). Day 0 of pregnancy was defined to start at 8 a.m. on the day a copulatory plug was observed following overnight breeding. The rats received food (Purina Lab Chow) and water *ad libitum* and were provided with artificial lighting on a 14 h light/10 h dark circle; room temperature was kept 21°C. On the day of sacrifice, the dams were anesthetized using nitrous oxide and halothane and then exsanguinated. The crown-rump lengths, somite numbers and protein content of the embryos at the stages studied are given in Table 1. The cephalic neural tube was generally closed by round 11 a.m. on day 10.

The culture method for these studies on day 10, 11 and 12 was New's raft technique (New, 1967) whereby three embryos are affixed to a coverslip raft in each circulator and the medium circulated past them. The attachment by spreading Reichert's membrane and parietal yolk sac on the raft, transfer into the glass circulator and the control of the rate of gas delivery were technically very difficult. The cleaning, preparation and maintenance of the circulators and associated apparatus was time-consuming and tedious, as was anchoring and installing the embryos. The fixed position of the embryos on rafts allowed heart rate recording using

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TABLE 1

GROWTH MEASUREMENTS AND GLUCOSE UTILIZATION FOR SOMITE STAGE RAT EMBRYOS

Age in days	Crown-rump length (mm) (mean±SE)	Somites (mean±SE)	Protein (μg) (mean±SE)	Glucose utilized (μmoles per gm protein per hour)
9.5-10	–	0-8.8±0.3	21±5.9	852±50
10.3	2.0±0.05	11.3±0.3	45±2	731±91
11	3.19±0.05	25.3±0.2	236±5	474±32
12	5.46±0.13	35.3±0.5	730±43	312±39

a laser beam image on a photomultiplying cell in a device called Plasmom (Robkin and Shepard, 1972). The roller tube method which was subsequently developed (New *et al.*, 1973) is much simpler requiring only that the embryos be added to a small amount of medium in pre-gassed media. The latter method was used for the day 9 observations which were carried out over a 17 hour period. The day 10, 11, and 12 cultures were exposed for 3 h.

For the experiments on days 10, 11 and 12 uniformly labeled ^{14}C glucose was added to a known amount of medium containing rat serum and Hank's and at the end of the incubation the incorporation of ^{14}C glucose into the embryos, embryonic membrane, lactate and CO_2 produced were measured using a liquid scintillation counter (Tanimura and Shepard, 1970). For the rat embryos grown from day 9.5 to day 10 the glucose and lactate concentrations were measured chemically (Shepard and Park, 1994) but the incorporation into the embryo membranes and CO_2 production were not measured.

The results as given in Figure 1 indicated that the utilization of glucose in μmoles per gm of protein per hour dropped very dramatically between day 9 and 12 of gestation. Most of the decrease in utilization was associated with a reduction in the proportion of lactate produced (from about 90 to 67%). A slight increase in the embryonic incorporation of ^{14}C glucose and CO_2 produced occurred with increasing maturation.

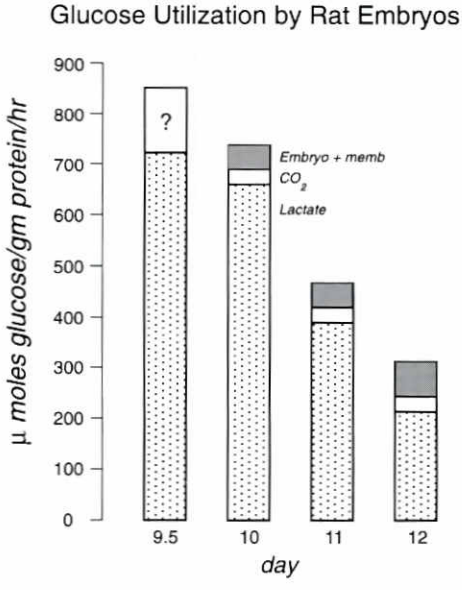


Fig. 1. *In vitro* utilization of glucose by rat embryos calculated from studies using uniformly-labeled glucose ^{14}C on days 10, 11 and 12. *For day 9.5 chemical analysis was used to determine glucose concentration drop and lactate increase. Note sharp drop in lactate production after day 10.*

Role of amniotic cavity in uptake of glucose and embryonic axis rotation

The problem of how glucose enters the embryo particularly before embryonic circulation commences was impossible to study until a micromethod for glucose assay was available. By using glucose oxidase indicator paper and precalibrated microbore glass pipettes, we were able to collect and analyze volumes of 1-3 μl (Park and Shepard, 1994). The volume of the amniotic fluid was determined by adding the embryo and membranes to a small piece of parafilm. The total weight was subtracted by the weight found immediately after opening the amnion and removal of the fluid with absorptive paper. Any fluid component of the exocoelomic cavity would have been included and been measured. We were unable to accurately study day 9 embryos with these techniques. Further details have been published by Park and Shepard (1994).

Changes in the volume of amniotic fluid during the development of the rat embryo/fetus are given in Table 2.

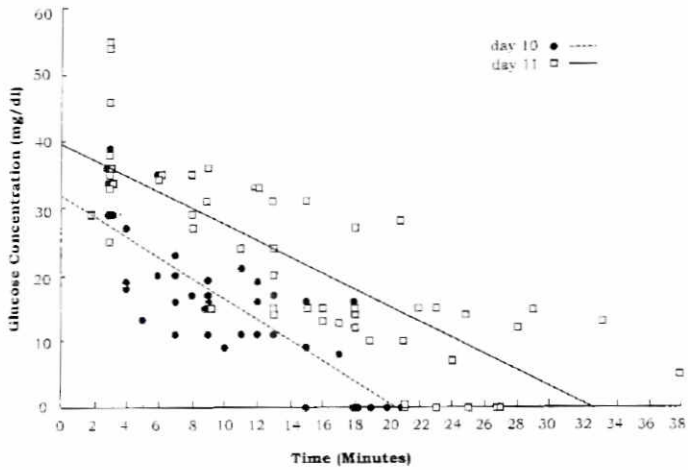


Fig. 2. Amniotic glucose depletion on days 10 and 11. *The regression for day 10 is y= 32.1-1.6 x and for day 11, y= 39.8-1.2x where y is the concentration of glucose and x is minutes. (From Park and Shepard, 1994).*

Between days 10 and 18, mean amniotic glucose concentration varied between 27-33 mg per dl until day 20 when it dropped to 16.8. We noted that glucose concentration dropped in amniotic fluid on days 10 & 11 over a 40 minute time period and studied glucose depletion from the amniotic cavity of embryos stored at 0 and 38°C. Over the time course of 14-32 min the glucose disappeared at 38°C (see Fig. 2) but was not changed at 0°C. Using these disappearance rates we calculated that 323 and 248 μmoles per glucose of protein per hour were utilized from the amniotic cavity by the embryos and membranes on day 10 and 11. This represents a substantial portion of the total glucose utilized; 44% on day 10 and 54% on day 11.

Based on the above disappearance rates we wondered if a 2 hour storage in glucose-free Hanks' would affect the subsequent *in vitro* growth of day 9.5 embryos. The embryos grew for 20 h in regular media at the same rate as controls stored for 2 h in regular Hanks' (100 mg/dl of glucose) at room temperature.

The content or weight of the amniotic cavity increases 7-fold between day 10 and 11 and this has a significant primary or secondary role on the axis rotation of the embryos. Day 10.3

embryos when removed from the restraint of the amnion rapidly straightened or partially converted their axes from dorsal to ventral flexion. In Figure 3 are pictured three embryos before and about 5 min after removal of the amnion. In another few minutes the two at the bottom were completely converted and the one at the top partially converted.

Role of the neural plate microvillus in uptake of glucose

A striking elongation of the microvilli of all the neural plate epithelium was observed at room temperature while viewing some day 10 rat embryos; serendipitously, it was determined that the cause was glucose since it was found only after exposure to Hanks' solution but not in Ringer's solution which contains no glucose. Subsequently, the previous observations of Lange *et al.* (1990) and Lange and Brandt (1990) on tissue culture cells were useful in understanding the phenomenon (see Discussion).

Day 10 rat embryos were dissected out of their membranes and exposed at room temperature (21-23°C) for 40 minute periods to glucose-free Hanks' containing various substituted hexoses, cytochalasin D, 2-deoxyglucose and 3-0-methylglucose. Exposure to 10 ng/dl of cytochalasin D was also studied. The microvillar reaction to glucose was studied at 38°C after 40 min in Hanks' or for 3 h or 17 h in whole embryo culture starting on day 9.5. The glucose utilized and lactate produced were studied in the media by chemical measurement at the start and finish of the culture. The starting glucose level was adjusted by using dialyzed rat serum with added B-vitamins and varying amounts of glucose (Shepard *et al.*, 1993; Shepard and Park, 1994).

The electron microscopic scanning was performed using a Field Emission Scanning Electron Microscope (JSM 6400F JEOL) after fixation in phosphate buffered glutaraldehyde, dehydration, critical point drying and sputter-coating with gold-palladium (Shepard and Park, 1994; see Fig. 4).

At room temperature the microvilli increased in length by about 10-fold (from 100-600 to 2,000-5000 nm) during a 40 minute incubation (Figs. 5-6). After a subsequent 40 minute exposure to glucose-free media the microvilli returned to their original length.

The resulting glucose-dependent mat obscured the underlying cells and at 35,000 magnification or above a right-handed helical surface pattern was noted (Fig. 8). A similar elongation was found in microvilli on the neural plate of day 9 mouse embryos but the microvilli were not as numerous and each neural cell contained a central cilium unlike the comparable rat embryo.

TABLE 2

GROWTH OF THE RAT EMBRYO/FETUS AND AMNIOTIC VOLUME AND GLUCOSE CONCENTRATION (MEAN±STANDARD ERROR)

Day	CR length (mm)	Embryo weight (mg)	Total protein (μg/embryo)	Amniotic volume (μl)	Amniotic glucose (mg/dl)
10	1.8±0.1	0.9±0.2[1]	46.7±3.2[1]	3.7±0.3	27.1±1.6
11	3.5±0.1	5.7±0.4[1]	386±12[1]	28.9±1.5	33.7±1.3
12	6.5±0.1	26.9±0.9[1]	1680±62[1]	80.0±3.2	33.7±1.3
14	11.4±0.1	163±5	11003±420	244±6	27.1±1.9
16	15.5±0.1	471±10	61849±2737	420±13	33.5±1.2
18	24.0±0.3	1636±28	–[2]	651±11	29.8±1.2
20	35.7±1.0	4339±49	–[2]	609±21	16.8±1.7

[1]Included membranes; [2]data not available.

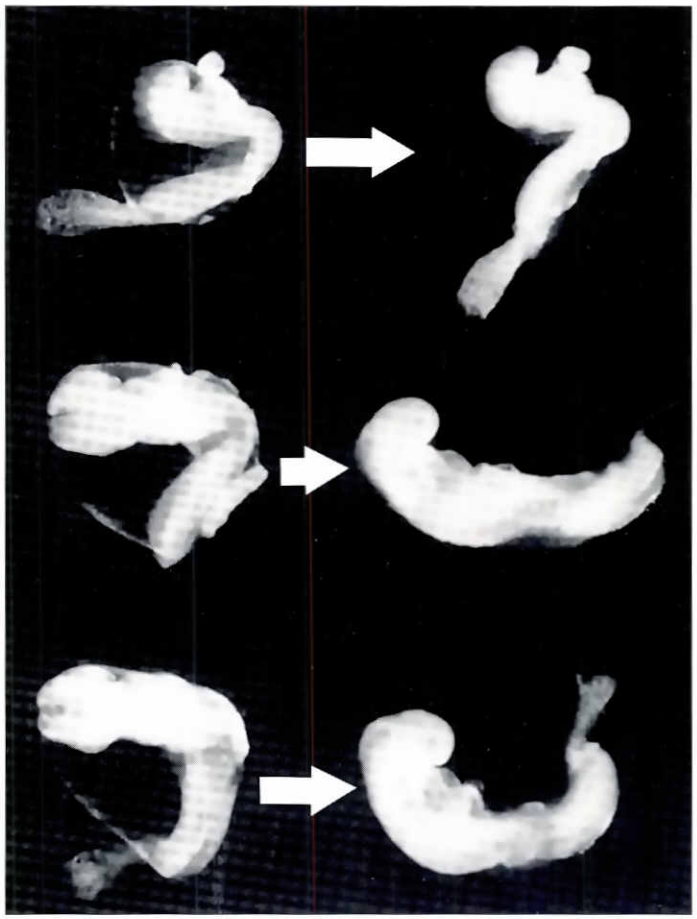


Fig. 3. Three day 10 embryos before (left) and after (right) removal of the amnion. *Time interval was about 5 min.*

During short-term exposures at room temperature fructose, d-galactose, l-glucose, 2-deoxyglucose and 3-0 methyl glucose were substituted for the glucose in Hanks'. Of these, only 2-deoxyglucose caused elongation of the microvilli. The matting produced by 2-deoxyglucose did not disappear following another 40 minute exposure to glucose-free Hanks'. When cytochalasin D (10 ng/dl) was added, the microvilli became markedly ballooned (Fig. 9).

Since no elongation of microvilli was observed at 38°C in Hanks' after exposure for 40 min or 2-3 h in culture, we initiated studies on day 9.5 for 17 h in whole embryo culture (Shepard and Park, 1994). A glucose-dependent increase in matting was found at ending concentrations of about 50 mg/dl and above (Fig. 10). Partial matting consisting of mats in parts of the mesencephalon but shorter microvilli in the prosencephalon and rhombencephalon occurred at the ending glucose concentrations of 40-70 mg/dl. No matting was seen in embryos grown in medium ending in concentrations of 26 and 29 mg/dl. In the studies with lower concentrations of glucose it was necessary to add extra glucose during the culture to prevent the glucose from dropping below 25 mg/dl and death of the embryos. The growth of the embryos was not decreased in the cultures ending with glucose as low as 26 or 29 mg/dl. The glucose utilized and lactate produced did not change significantly with

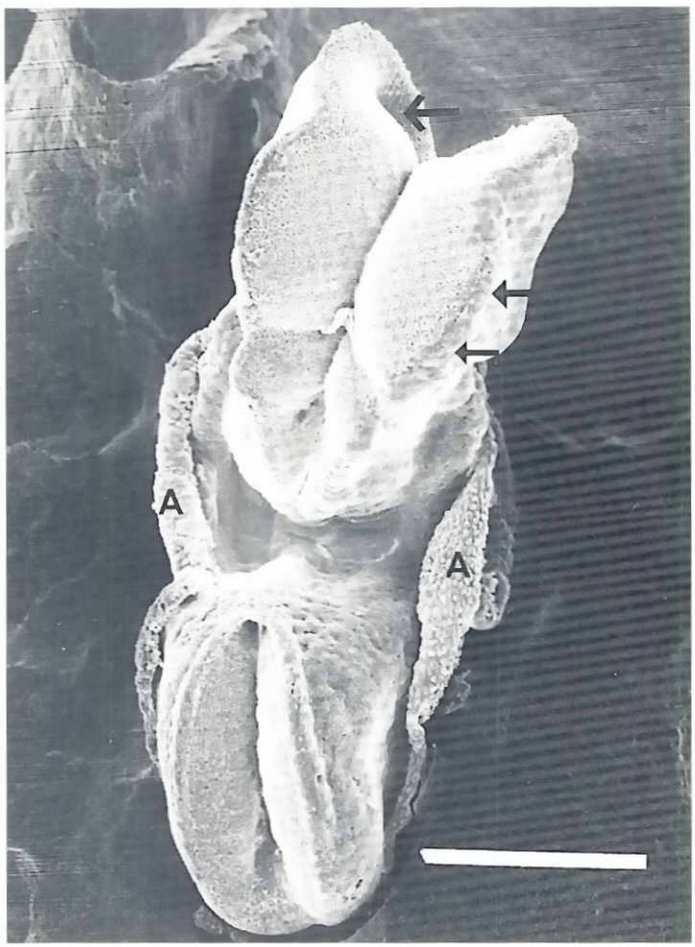


Fig. 4. Day 10 rat embryo. *Amnion (A). The cervical neural tube has closed. Arrow at top indicates optic vesicle. Arrows below indicate smooth area between skin and neural ectoderm. Bar, 250 μm. (From Shepard et al., 1993). Most of the studies of microvillus lengthening were done at this stage.*

ending glucose concentrations between 29 to 114 mg/dl. Pyruvate substituted for glucose in the cultures did not support full growth and the embryos died after 3-5 h.

The microvilli inside the closed neural tube in day 10, 11 and 14 rat embryos were only 50-100 nm in length (Fig. 7). The measurements of the glucose concentration in the neural tube fluids were 10.4 (2.9±SE) and 9.7 (3.7 mg/dl for day 11 and 14 embryo respectively.

Discussion

Uptake and utilization of glucose

Previously published studies have shown that glycolysis at early somite embryo stages is primarily anaerobic (Shepard *et al.*, 1970; Tanimura and Shepard, 1970; Neubert, 1973; Clough and Whittingham, 1983; Ellington, 1987a). Providing a source of nucleic acids, the pentose phosphate shunt is also very active during these period (Clough and Whittingham 1983; Ellington, 1987a). The drop in glucose utilization from 852 μmoles/gm/h on day 9.5-10 to 312 umoles per gm of protein per hour is associated with a similar drop in lactate production as glycolysis via the Embden-Myerhof pathway (anaerobic) is switched to the Kreb's cycle and terminal electron transport system (aerobic). At early somite stages, there is no vascular system to deliver oxygen and at about day 10.5 embryonic circulation begins to make more oxygen available. To put this utilization rate into perspective, the rate in rapidly beating adult rat hearts is only 267 to 515 μmoles glucose per gm per hour (Vahouny *et al.*, 1966). In our *in vitro* culture system optimal oxygen concentration in the gas phase increases from day 9.5 when only 5% oxygen is used. On day 10 we use 20% oxygen and on day 11 and thereafter 95% oxygen is necessary. On day 9 even 20% oxygen is toxic to the embryos. Studies on isolated mitochondria from day 10-14 have shown increases in DPNH oxidase, cytochrome C oxidase, succinic dehydrogenase and mitochondrial ATP-ase, and these changes were paralleled by striking changes in the number and structure of the cristae of heart mitochondria (Mackler *et al.*, 1971). Altogether there is a striking association between the switch from anaerobic to aerobic glycolysis and increasing activity of the Kreb's cycle and terminal electron transport activity, together with structural maturation of the mitochondria and the requirement for higher oxygen concentrations in culture.

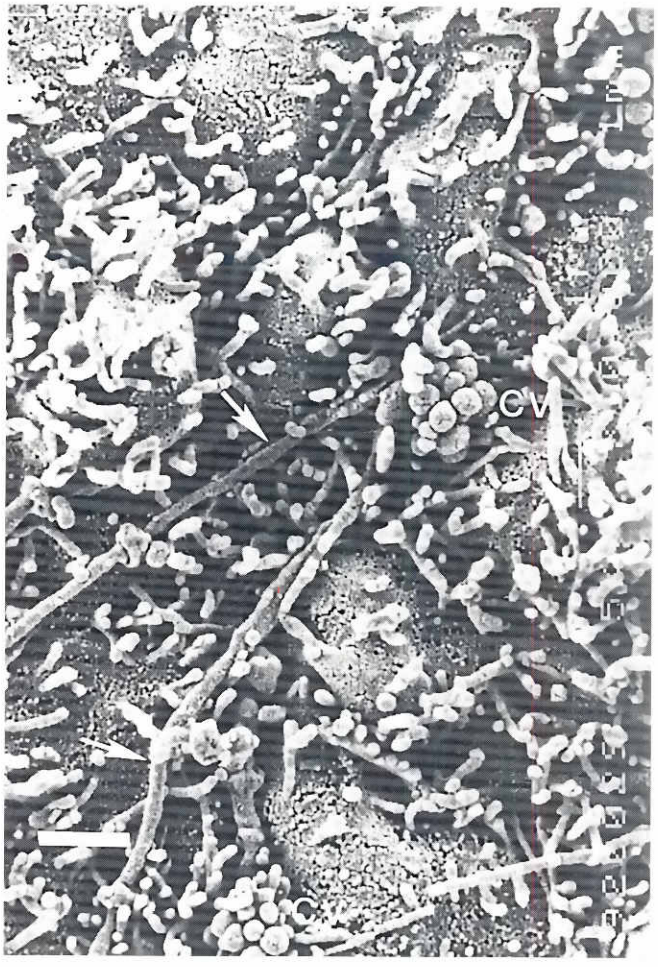
Since glucose is the major source of energy during early embryogenesis, any interruption in its transport or utilization could initiate or be part of the cascade of events that lead to eventual congenital defects. Even more specifically one might expect that in the very early somite stages agents which affect anaerobic glycolysis would be more liable to produce congenital defects than those affecting the terminal electron transport system. For example, during the anaerobic glycolysis period, carbon monoxide which inactivates the cytochrome elements of the terminal electron transport system would be expected to have no effect. Indeed, it has been demonstrated that carbon monoxide exposure does not alter early somite embryonic growth (Robkin *et al.*, 1976). Hiranruengchok and Harris (1995) found decreased glycolysis and pentose phosphate shunt pathway activity was produced by diamide exposure in organogenesis-stage rat conceptuses studied *in vitro*.

The transfer of sufficient oxygen into the late somite embryo under *in vitro* conditions is so far not thoroughly understood. A specific carrier for oxygen has been identified in the placenta (via cytochrome p-450) but the rate constants do not support the idea that the transfer is of sufficient extent to support embryos or fetuses (Burns and Gurtner, 1973).

Role of amniotic cavity in uptake of glucose and embryonic axis rotation

After day 8 when the amnion is formed in the rat embryo, the amniotic fluid is an important environment for the rapidly developing nervous system because the neural plate is always directly exposed to amniotic fluid. The amniotic fluid first became collectible on day 10.3; about 3 h later (11 a.m.) the cranial part of the neural tube closes and embryonic circulation begins. Based on the disappearance of glucose at 38°C from day 10 amniotic cavities we calculated that 323 μmoles of glucose per gm of protein per hour was removed. This represents about 44% of the glucose used by embryo and membranes at this stage (Park and Shepard, 1994). Thus we believe the entry of glucose through the amniotic cavity is a substantial source of energy for neural plate and embryo morphogenesis.

There is ample evidence for the presence of glucose transporters in somite stage rat and mouse embryos. Glucose transporters



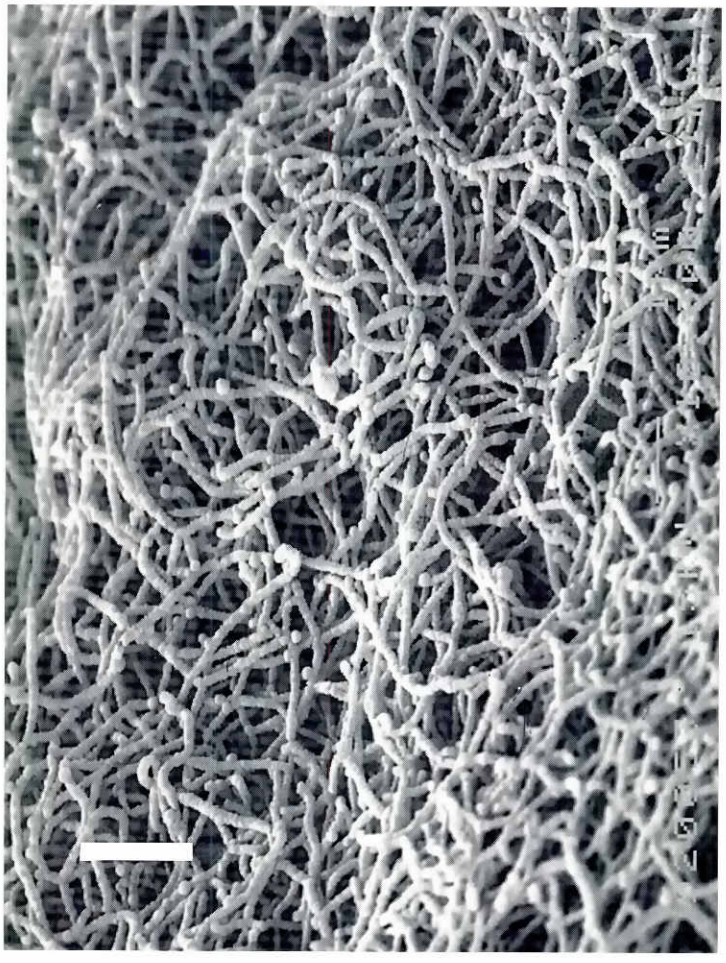


Fig. 5 (left). Surface of the neural plate fixed immediately after removal from the uterus, no glucose exposure. *The microvilli are mostly short (100-600 nm), but occasionally there are very long ones (arrows). CV marks the clumps of vesicles. Bar, 1 μm. (From Shepard et al., 1993).*

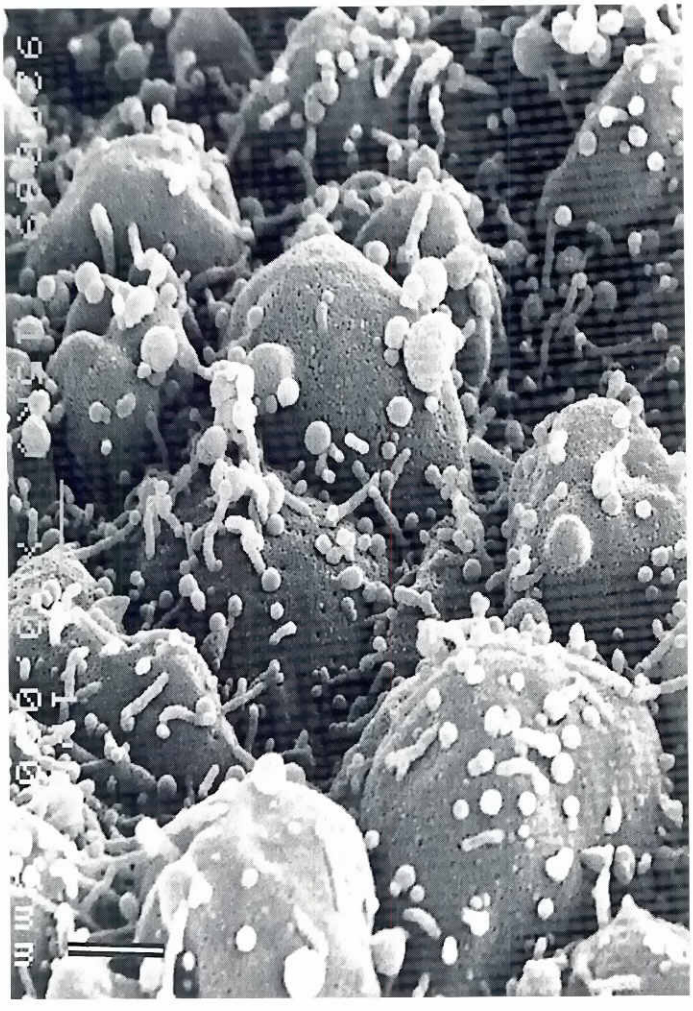
Fig. 6 (right). Surface of the neural plate after 40′ exposure to Hanks' with glucose. *The microvilli are now 2,000 - 5,000 nm length and can be seen to have segments. Bar, 1 μm. (From Shepard et al., 1993).*

1, 2 and 3 have been identified in mouse embryos but glucose transporter 4 was not found (Hogan *et al.*, 1991; Smith and Gridley, 1992). In the rat embryo glucose transporters 1 and 3 have been identified and localized (Takao *et al.*, 1993; Trocino *et al.*, 1994). In both species glucose transporter 1 is localized in the neuroepithelium, amnion and yolk sac. In the rat, embryo glucose transporter 3 is expressed in endoderm, amnion and yolk sac (Takao *et al.*, 1993). In the mouse, glucose transporter 3 is expressed in the membranes and in patches of skin surface ectoderm (Smith and Gridley, 1992). If the hypothesis about the tip of the microvillus being the entry point of glucose is true (see later discussion), then glucose transporter 1 should be localized on that part of the microvilli.

In these studies, starting on day 10.3, we were able to estimate the amniotic fluid volume by the differences in weight of the embryo and membranes before and after draining off the fluid. The amniotic fluid volume on day 15 rat is reported by Barker in Adolf (1967) to be similar to that reported here. We believe the 7-fold increase in volume between day 10 and 11 is an important factor permitting the embryo to rotate its axis. Invariably we observed that when the amnion was removed from day 10 embryos; they rapidly straightened and partially or completely converted their axes from dorsal to ventral flexion (see Fig. 3). Deuchar and Parker (1975) have previously described the effect of the amnion release on axis rotation of rat embryos and based on studies after transecting the trunk they suggest that somatic muscles also participate in body turning. McCafferty (1955) has summarized amniotic fluid volume changes during development in mammals. If the amniotic fluid volume should fail to increase during early somite stages the resulting axis distortion might prevent neural tube closure via a mechanism similar to that described by van Straaten *et al.* (1993) for the caudal neural tube. Adhesions between the tail area and cervical neural tube have been observed in embryos grown *in vitro* and exposed to teratogens (Klein *et al.*, 1982).

Neural plate microvilli and uptake of glucose

The control of membrane-limited glucose uptake in distinction to simple diffusion uptake has been linked in tissue culture to the presence of surface microvilli (Lange *et al.*, 1989, 1990; Lange and Brandt, 1990). The decreased glucose concentration within the



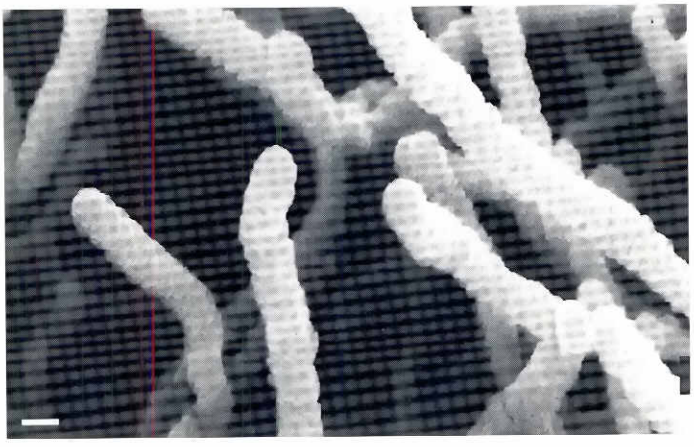


Fig. 7 (left). The inner surface of the closed neural tube on day 11. *Most of the microvilli are extremely short measuring 50-100 nm with occasional lengths of 400 nm. Some of the short microvillar tips measured up to 400 nm in width. Bar, 1 μm. (From Shepard et al., 1993).*

Fig. 8 (right). The right-handed helical pattern can now be seen on the surface of the microvilli exposed to Hanks' with glucose for 40 min. *Terminal portions appear to have smooth edges. Bar, 100 μm. (From Shepard et al., 1993).*

cells after this membrane-limited uptake is referred to as the glucose curb. A concept has been developed that the tip of the microvillus is the entrance compartment for glucose and the shaft sets up a diffusion barrier into the cytoplasm (Lange *et al.*, 1989, 1990). The passage of glucose from the entry compartment is controlled in part by the length of the microvillus shaft (Lange *et al.*, 1989; Shepard *et al.*, 1993; Shepard and Park, 1994). With lengthening of the microvillus shaft a diffusion barrier to glucose is increased. Further support for the hypothesis that the microvillar length controls uptake of glucose was found in the neural tube after closure when the low glucose concentrations are associated with very short microvilli (Shepard *et al.*, 1993). Apparently the microvilli of the living cells of the closed neural tube are in this state to increase glucose uptake once they lose access to amniotic fluid.

Based on our findings, there may be two separate processes for microvillar lengthening following glucose exposure. One is a rapid response which occurs at 21-23°C and the other at 38°C takes 17-24 h in cultured rat embryos or tissue culture. Most enzymatic reactions are more rapid at 38°C than at 21-23°C, but studies of the kinetics of actin polymerization have shown that there is no appreciable acceleration in the reaction between 25°C and 35°C (Kinosian *et al.*, 1991). Perhaps, the conditions leading to actin polymerization at 21-23°C involve the cytoplasmic end of actin filaments while at the 38°C the elongation could be primarily from the distal or barbed ends. Another explanation for the more rapid microvillar elongation at room temperature might be depletion of phosphate in the microvillar compartment secondary to metabolic changes in the cellular compartment at lower temperature. The dynamics of actin polymerization and ATP hydrolysis have reviewed by Korn *et al.* (1987) and Carlier (1991).

The effects of low glucose concentration on the *in vitro* growth of rat and mouse embryos have been studied. Akazawa *et al.* (1987) and Ellington (1987b) have reported on the glucose requirements of cultured rat embryos. Ellington (1987b) cultured day 9.5 embryos starting at concentrations of 81 or 61 mg/dl for 24 or 48 h and reported that glucose uptakes were reduced when this glucose concentration in media fell below 48 mg/dl. Decreased growth and dysmorphic fetuses were found after 48 h in culture. Reversal of the deleterious effects could not be achieved by reconstitution of normal concentrations of glucose after 24 h. Akazawa *et al.* (1987) cultured day 9.5 embryos for the first 24 h in concentrations of glucose ranging from 30 to 80 mg/dl and then examined them after a further 24 h in culture media with 100 mg/dl of glucose. Somite numbers and crown-rump lengths were reduced compared to the controls grown continuously in media with 100-110 mg/dl glucose. Although the conditions and endpoints in these two experiments differed from our 17 h cultures, the requirements for glucose were somewhat comparable.

The day 8 mouse has been reported to be relatively more sensitive to hypoglycemia in experiments performed by Smoak and Sadler (1090). These investigators found malformations in embryos exposed to 60 mg/dl or less for 2 h followed by 22 h culture in normoglycemic medium (120-150 mg/dl). We found no ill effects from exposure to Hanks' without glucose for 2 h followed by 19 h of culture. Does this difference indicate that the mouse is a species more sensitive to hypoglycemia? This question might be answered

Fig. 9. Neural epithelium of embryos exposed for 40 min at 21-23°C to glucose-free Hanks' containing cytochalasin D (10 ng/dl). *The microvilli are short and ballooned. One microvillus is unaffected and stretches between cells (arrow). Bar, 0.5 μm. (From Shepard et al., 1993).*

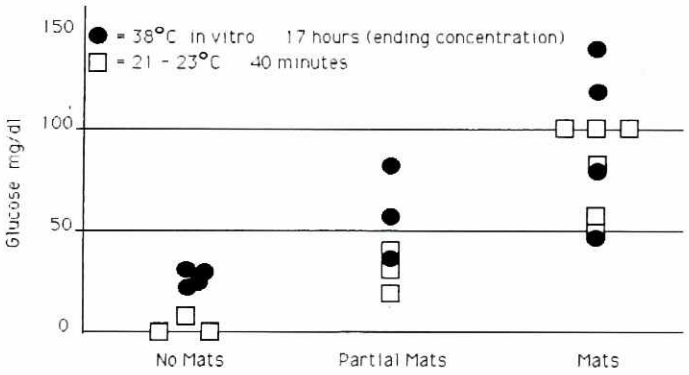


Fig. 10. Plot to show relationship between final glucose concentration and state of matting in 23 experiments *for embryos grown 17 h in whole embryo culture (●) or embryos exposed for 40 min at 21-23°C (□). For the in vitro culture R^2= 0.66 (df= 9, p<0.01); for the 40 min exposure at 21-23°C R^2= 0.81 (df 10, p<0.01). (From Shepard et al., 1993).*

by growing presomite rat and mouse embryos at glucose concentrations of 30-40 mg/dl. As described in the Results, the mouse, unlike the rat, has more sparse microvilli on the surface of neural cells and short cilia are present.

There is general agreement that the lengthening of microvilli is associated with stacking of actin filaments at the distal end (Alberts *et al.*, 1989). ATP is involved in this polymerization and the kinetics have been studied (Kinosian *et al.*, 1991). Using 40 minute exposures at room temperature we found no increase in the length of the microvilli after exposure to 1-glucose, fructose, galactose or pyruvate, all of which are not phosphorylated. On the other hand, 2-deoxy-d-glucose, which is absorbed and phosphorylated, caused lengthening of the microvilli in a similar way to that seen with d-glucose. The phosphorylated 2-deoxy-d-glucose does not enter the glycolytic pathway and therefore accumulates, and in some way induces microvillus lengthening. 3-0-methyl glucose which is absorbed but not phosphorylated did not produce microvillar lengthening. Thus phosphorylation seems to play an important role in lengthening of the microvillar shafts.

Cytochalasin D is known to disrupt microfilaments and in the concentrations used here prevents neural tube closure in whole rat embryo culture (Fantel *et al.*, 1981). Cytochalasin binds to the growing ends of actin and thereby causes disruption of microfilaments (Franki *et al.*, 1992).

The role of microvillar lengthening in glucose uptake is of more than a physiologic interest. If glucose concentration can significantly alter the cell membrane of general epithelia, other absorptive functions might also be affected. For instance, in uncontrolled hyperglycemia a microvillar mat might interfere with the uptake of amino acids or lipids and lead to some of the degenerative changes seen with diabetes. If the cells of the immune system are involved, the microvillar alterations of hyperglycemia could interfere with their activities. These and other hypotheses should be explored.

In summary, there is little doubt that glucose plays a significant nutritional role in early somite embryos. The high glucose utilization of anaerobic glycolysis drops as the activity of the Kreb's cycle and terminal electron transport pathway increase. Concurrently, maturation of mitochondrial cristae and dependence on oxygen supply are taking place. The neuroepithelium of the early somite rat embryo responds *in vitro* during culture by microvillar lengthening when exposed to glucose levels of 50 mg/dl or more. At lower glucose concentrations both in whole embryo culture and inside the closed neural tube the microvilli are shorter. Lengthening of the microvilli at room temperature is produced only by d-glucose and 2-deoxyglucose, two hexoses that are absorbed and phosphorylated. Cytochalasin D which disrupts actin polymerization causes ballooning of the microvilli.

The amniotic cavity is one major portal of entry for glucose during the early somite embryo stage. The 7-fold increase in volume of the amniotic cavity after day 10 allows the rat embryo to convert its axis from dorsal to ventral flexion.

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