In vitro analysis of glucose metabolism and embryonic growth in postimplantation rat embryos

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Summary

The glucose metabolism and embryonic development of rat embryos during organogenesis was studied using embryo culture. Glucose uptake and embryonic growth and differentiation of 10-5-day explants (embryos + membranes) were limited by the decreasing glucose concentration, but not the increasing concentration of metabolites, in the culture media during the second 24 h of a 48 h culture. No such limitations were found on the embryonic development of 9-5-day explants during a 48 h culture although glucose uptake was slightly reduced at very low concentrations of glucose. From the head-fold stage to the 25-somite stage of development, glucose uptake was characteristic of the stage of development of the embryo and not the time it had been in culture.

Embryonic growth of 9-5-day explants was similar to that previously observed in vivo. Glucose uptake by 9-5-day explants was dependent on the surface area of the yolk sac and was independent of the glucose concentration in the culture media (within the range of 9.4 to 2.5 mM). The proportion of glucose converted to lactate was 100% during the first 42 h of culture then fell to about 50% during the final 6 h. The protein contents of both the extraembryonic membranes and the embryo were dependent on the glucose uptake.

Key words: glucose metabolism, rat embryo, postimplantation.

Introduction

The growth and morphological development of rat embryos from the early head-fold to the 25-somite stage is extremely rapid and occurs at similar rates in vivo and in vitro (New, Coppola & Cockroft, 1976).

Embryo culture techniques have been used in the study of rat and mouse embryonic glucose metabolism during this period of rapid growth and differentiation and it has been shown that, in vitro, the major energy source of embryos at this stage of development is glucose (Cockroft, 1979) and that the energy is derived primarily from the glycolytic and pentose phosphate pathways (Clough & Whittingham, 1983; Cox & Gunberg, 1972a,b; Neubert, Peters, Teske, Köhler & Barrach, 1971; Shepard, Tanimura & Robkin, 1970; Spielmann, Meyer-Wendecker & Spielmann, 1973; Tanimura & Shepard, 1970). Rat embryos more advanced than the 25-somite stage showed a progressive decline in the ratio of glucose utilization to lactate production in vitro (Shepard et al. 1970).

Similar techniques have also been used to study aspects of carbohydrate metabolism under pathological conditions such as maternal diabetes or fasting (Deuchar, 1979; Cockroft, 1979, 1984; Ellington, 1980; Sadler, 1980; Sadler & Horton, 1983).

With this increasing use of culture techniques to study both normal and abnormal carbohydrate metabolism it is important to establish the extent to which embryonic metabolism in vitro is affected by parameters of the culture methods. Sanyal (1980) and Clough & Whittingham (1983) suggest that the accumulation of metabolites in the culture media could inhibit embryonic metabolism. Glucose concentrations in the media decrease progressively during the course of the culture (Sanyal, 1980) and could also limit embryonic growth.

Glucose uptake from the culture medium presumably occurs at the surface of the visceral yolk sac which completely envelopes the embryo and acts as the functional placenta until the formation of the chorioallantoic placenta (Amoroso, 1952). There have been no detailed studies of glucose uptake.
across the visceral yolk sac but some of its placental functions have been illustrated by the accounts of the uptake and digestion of protein by the yolk sac (e.g. Beck, Lloyd & Griffiths, 1967; Freeman, Beck & Lloyd, 1981; Jollie, 1986).

The project described in this paper can be divided into two sections. The experiments in the first section were designed to assess the extent to which embryonic metabolism is influenced by parameters of the culture system. The second section contains a more detailed study in which the rates of glucose uptake and lactate secretion are correlated with the surface area of the visceral yolk sac and the protein content and morphological development of the embryo and extraembryonic membranes.

Materials and methods

Animals and techniques

Embryos and serum were obtained from CFHB rats (Interfauna, Wyton, UK). Females were caged with males overnight and those females with sperm in the vagina the following morning were regarded as half a day (0-5 day) pregnant at noon that day. Embryos and their membranes (explants) were dissected from 9-5 to 10-5-day pregnant rats as previously described (New, 1973). The parietal yolk sac was opened but the remaining membranes left intact. The explants were placed in culture bottles containing immediately centrifuged, heat-inactivated serum (Steele & New, 1974) containing 6 µg ml\(^{-1}\) penicillin and 10 µg ml\(^{-1}\) streptomycin. In the first two series of experiments four explants and 5 ml of serum were placed in each bottle, in the third series (in which most of the cultures were for a shorter duration) the volume of serum was reduced to 4 ml. The culture bottles were attached to a 'Rotator' (New & Cockroft, 1979) and gassed with a gas mixture containing 5, 20 or 40 % oxygen, depending on the stage of development of the embryos, and 5 % carbon dioxide and nitrogen (New et al. 1976).

At the termination of the cultures, the intact explants were removed from the media and the heart beat and yolk sac circulation of each were immediately assessed. Two yolk sac diameters were measured, the first from the centre of the ectoplacental cone to the point opposite on the antimesometrial surface of the yolk sac and the second at right angles to this across the broadest part of the yolk sac. The embryos were then removed from their membranes and their crown–rump lengths measured and the number of somites counted. The embryos, and in some experiments the membranes (i.e. the amnion, visceral yolk sac, allantois and the developing choiorallantoic placenta) were individually assessed for protein content by the method of Lowry, Rosebrough, Farr & Randall (1951). The culture media were assayed for glucose and lactate content by enzymatic, colorimetric techniques using commercial kits (diagnostic kits numbers 510 and 826-UV respectively; Sigma, Poole, UK).

Experiments

Expt 1: normal glucose uptake by explants in culture

9-5- and 10-5-day explants were cultured for 48 h, the serum glucose concentrations were assayed at the start of the culture then at regular intervals until its termination. The explants were assessed for embryonic growth and development at the end of the culture.

Expt 2: effects on glucose uptake and embryonic growth and development of supplementing the serum glucose or changing the serum

10-5-day explants were cultured for 48 h during which time the glucose in the culture media was assayed regularly. After 24 h of culture some of the bottles had glucose added to return the serum glucose concentration to that at the start of the culture, others had the serum changed for an equal volume of fresh serum. The serum in the control bottles was neither changed nor supplemented with glucose. 9-5-day explants were cultured for 48 h either with no adjustment to the glucose concentrations or with the glucose supplemented after 18, 30 and 42 h of culture.

Expt 3: glucose uptake, lactate production and the growth of embryos and embryonic membranes in culture

9-5-day explants were cultured for 12, 24, 36, 42 or 48 h. Glucose and lactate concentrations in the serum were assayed before and after culture. The development of the explants at the end of the culture was assessed as usual and the surface area of the yolk sac was calculated as for the surface area of a sphere with a diameter equal to the mean of the two measured diameters.

Analyses of results

The results of experiment 2 were analysed by the Students' r-test to test for the significance of the effects of the different treatments on glucose uptake, embryonic size and development.

The results of experiment 3 were analysed as linear regressions of log.-transformed variables in order to establish allometric relationships between the variables.

Results

Expt 1: normal glucose uptake by explants in culture

After 48 h in culture, the embryos explanted at 9-5 and 10-5 days had crown–rump lengths of 3-0 ± 0·1 and 4-0 ± 0·1 mm, respectively, and an average of 24·6 ± 0·2 or 36·4 ± 0·3 somites each.

Fig. 1 shows the rate of glucose depletion from the culture medium and the calculated values for glucose uptake per embryo over the 48 h culture period. (Glucose concentrations in serum from control bottles without explants did not change during the course of the culture. It was therefore assumed that the glucose depletion from the media in the presence
Glucose metabolism and embryonic growth

Fig. 1. The fall in serum glucose concentrations (---) and the calculated glucose uptake per embryo (----) during the course of 48 h cultures of (A) 9-5-day explants and (B) 10-5-day explants. Each point on the serum glucose concentration curve represents the mean (±s.e.) of serum glucose in 10 culture bottles, each containing 4 embryos. The glucose uptake per embryo is the mean (±s.e.) of the 40 embryos.

of explants reflected glucose uptake by the explants. In each experiment, glucose uptake per explant was calculated from the assayed serum glucose concentrations.) The rate of glucose uptake by 9-5-day explants appeared to increase throughout the culture period even though the glucose concentration in the medium had fallen to about 2-5 mm by the end of the culture. The rate of glucose uptake by 10-5-day explants increased during the first 24 h but showed a decrease thereafter which was especially marked during the final 12 h of culture, by which stage the serum glucose concentration was under 1-5 mm.

In order to establish whether the rate of glucose uptake was characteristic of the equivalent age of the explant (i.e. the age of the embryo at explantation plus the time it had been in culture) or whether it was influenced by the length of time the explant had been in culture, the glucose uptake by 9-5- and 10-5-day explants of the same equivalent age was compared (Fig. 2). Between 10-5 and 11-5 days the glucose uptake was characteristic of the equivalent age of the embryos and appeared not to be modified by the length of time that the embryo had been in culture.

Expt 2: the effects on glucose uptake and embryonic growth and development of supplementing the serum glucose or changing the serum

(a) 10-5-day explants
The glucose uptake was significantly higher by explants cultured under a regime including a serum change (P < 0.001) or glucose supplement (P < 0.01) after 24 h than by control explants which had neither fresh serum nor added glucose during the culture (Fig. 3A). At the termination of the culture the control embryos had shorter crown–rump lengths and contained significantly less protein (P < 0.01) than embryos from either of the experimental groups (Table 1A). There was no significant difference in the stage of development (as assessed by somite numbers) between embryos in the control and the experimental groups. The glucose uptake and embryonic growth and development were similar in the two experimental groups. Additions of glucose to the serum appeared to be no less beneficial than a serum change after 24 h.

(b) 9-5-day explants
Although the glucose uptake was higher in the explants cultured in media in which the glucose was
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Table 1. The development of 10-5 and 9-5-day explants after 48 h in culture with or without addition of glucose or a serum change during the 48 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of embryos</th>
<th>Number of embryos with Heart beat</th>
<th>Yolk sac circulation</th>
<th>Crown–rump length (mm ± S.E.)</th>
<th>Somites (± S.E.)</th>
<th>Protein content (µg ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) 10-5-day explants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>4–0 ± 0.1</td>
<td>34.2 ± 0.6</td>
<td>514 ± 28</td>
</tr>
<tr>
<td>Glucose supplemented</td>
<td>40</td>
<td>11</td>
<td>9</td>
<td>4.7 ± 0.1*</td>
<td>34.6 ± 1.4</td>
<td>631 ± 35*</td>
</tr>
<tr>
<td>Serum changed</td>
<td>40</td>
<td>1</td>
<td>0</td>
<td>4.3 ± 0.1</td>
<td>35.0 ± 0.8</td>
<td>632 ± 38*</td>
</tr>
<tr>
<td>(B) 9-5-day explants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>19</td>
<td>16</td>
<td>3.2 ± 0.1</td>
<td>25.8 ± 0.2</td>
<td>188 ± 7</td>
</tr>
<tr>
<td>Glucose supplemented</td>
<td>40</td>
<td>15</td>
<td>12</td>
<td>3.1 ± 0.1</td>
<td>25.8 ± 0.2</td>
<td>196 ± 5</td>
</tr>
</tbody>
</table>

*Significantly different from the values for control explants P < 0.01 (Student's t-test).

Table 2. The development of 9-5-day explants after 12, 24, 36, 42 or 48 h in culture

<table>
<thead>
<tr>
<th>Number of embryos with</th>
<th>Number of embryos</th>
<th>Heart beat</th>
<th>Yolk sac circulation</th>
<th>U*</th>
<th>C</th>
<th>Yolk sac surface area (mm² ± S.E.)</th>
<th>Crown–rump length (mm ± S.E.)</th>
<th>Somite number (± S.E.)</th>
<th>Protein content (µg ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours in culture</td>
<td>Number of embryos</td>
<td>12</td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>6.2 ± 0.2</td>
<td>0</td>
<td>18 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>13.1 ± 0.3</td>
<td>9.9 ± 0.6</td>
<td>31 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>24</td>
<td>24</td>
<td>1</td>
<td>4</td>
<td>24.4 ± 0.6</td>
<td>2.3 ± 0.0</td>
<td>74 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>31.7 ± 0.7</td>
<td>2.8 ± 0.0</td>
<td>116 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>40.0 ± 0.9</td>
<td>3.1 ± 0.0</td>
<td>175 ± 5</td>
</tr>
</tbody>
</table>

* U, embryonic posture, dorsally concave; U–C, stage of turning from the embryonic to the fetal position; C, fetal posture, dorsally concave.

periodically supplemented than in the control explants the differences were not significant at the 0.01 level (Fig. 3B).

There were no significant differences in the crown–rump length, somite number or protein content of the embryos in the two groups at the termination of the culture (Table 1B).

Expt 3: glucose uptake, lactate production and the growth of embryos and embryonic membranes in culture

The development of 9-5-day explants after 12, 24, 36, 42 and 48 h is shown in Table 2. The embryos developed from the head-fold stage to about the 24-somite stage of development during the 48 h culture period. Initially the embryos were dorsally concave (U-shaped) and lying at the surface of the egg cylinder. With further development they became enveloped by the visceral yolk sac, somites and heart tissue differentiated and, shortly after 10-5 days, the embryos rotated from the embryonic, U-shaped posture to the fetal, C-shaped, posture with the dorsal surface convex and the head projecting forward over the heart.

Table 3 shows the concentration of glucose and lactate, as assayed, in the culture media at the start of the culture and after 12, 24, 36, 42 and 48 h and the calculated values for the glucose uptake and lactate secretion by the explants over the total culture period. (During glycolysis, each molecule of glucose yields two molecules of lactate, hence, if all the glucose in a one molar glucose solution was metabolized to lactate, the resultant solution would contain 2 mol-lactate. By comparing the changes in the molarity of the glucose and lactate in a specific volume of medium during the culture period the percentage of glucose converted to lactate was calculated.) During the first 42 h of culture, 9-5-day explants converted all the glucose taken up to lactate. The equivalent figures for the explants cultured for 48 h give a

Fig. 3. The fall in serum glucose concentrations (——) and the calculated glucose uptake per embryo (----) during the course of 48 h cultures of (A) 10-5-day and (B) 9-5-day explants with or without a change of serum or glucose supplements during the culture. Each point represents the mean (± S.E.) of data from 10 bottles or 40 embryos. (●) no treatment; (O) glucose supplement; (■) change of serum. Standard error bars, Fig. 3A. (●), no treatment; (O), glucose supplement; (■), change of serum.
Table 3. Glucose uptake and lactate secretion by 9-5-day explants during cultures of varying lengths up to 48 h

<table>
<thead>
<tr>
<th>Hours in culture</th>
<th>Number of bottles (embryos)</th>
<th>Concentration in culture media (mM)</th>
<th>Calculated values for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose</td>
<td>Lactate</td>
</tr>
<tr>
<td>0</td>
<td>6 (24)</td>
<td>9.4 ± 0.2</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>6 (24)</td>
<td>8.6 ± 0.2</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>24</td>
<td>6 (24)</td>
<td>7.4 ± 0.3</td>
<td>7.7 ± 0.2</td>
</tr>
<tr>
<td>36</td>
<td>6 (24)</td>
<td>5.3 ± 0.3</td>
<td>11.3 ± 0.2</td>
</tr>
<tr>
<td>42</td>
<td>5 (20)</td>
<td>4.6 ± 0.2</td>
<td>13.5 ± 0.3</td>
</tr>
<tr>
<td>48</td>
<td>6 (24)</td>
<td>2.5 ± 0.3</td>
<td>15.3 ± 0.4</td>
</tr>
</tbody>
</table>

glucose-to-lactate conversion rate of 86%. Assuming that glucose uptake and lactate secretion during the first 42 h of the culture was the same as in those cultures terminated after 42 h, the conversion rate of glucose to lactate during the final 6 h of the 48 h culture was 53%. These calculations ignore the possibility of accumulation of glucose or lactate within the conceptus. Since both these substances are freely diffusible, it was assumed that the concentrations inside the conceptus and in the media would be very similar.

The surface area of the yolk sac was proportional to the embryonic protein to the 1.5 (±0.1) power at all stages except in the cultures terminated at 12 h in which the embryonic protein content was higher than would be predicted by the above result.

Glucose uptake per unit area of yolk sac increased slightly as the yolk sac became larger i.e. glucose taken up by the explants in culture was proportional to the surface area of the yolk sac to the power of 1.1 (±0.1) (Fig. 4A). Glucose uptake was independent of the glucose concentrations in the media within the range of these experiments; 9.4 to 2.5 mM.

Data from cultures ended at 24 h or later showed that the glucose taken up from the media was proportional to the total protein content of the explant to the power of 0.9 (±0.1) and to the protein content of the embryo to the power of 0.73 (±0.1) (Fig. 4B). The protein content of embryos removed from culture after 12 h was slightly higher than would be expected from the above correlation.

Glucose uptake, embryonic protein, membrane protein, somite number and membrane surface area were all strongly correlated with the time that the 9-5-day explant had been in culture (correlation coefficient for all parameters against time >0.97). Embryonic protein content and somite numbers during the last 24 h of culture were similar to the values observed in rat embryos of 10.5–11.5 days in vivo (New, 1973).

Discussion

With the interstitial implantation and the extensive decidualization characteristic of muridine development, postimplantation rat and mouse embryos are inaccessible for in vivo experimentation, and we are dependent on observations of embryos in vitro for much of our knowledge of embryonic development. The aim of the work described in this paper was to
discover the extent to which embryonic glucose metabolism is influenced by the conditions of the culture system and then, under optimum conditions, to study glucose metabolism and the growth of the embryos and their membranes.

In the initial experiments, embryos were cultured under conditions already shown to support embryonic growth and development very similar to that occurring in vivo. Under these conditions, glucose uptake was very consistent both between cultures of explants of the same age and between explants at the same stage of development even after differing periods in culture (Fig. 2).

In order to test the hypothesis that embryonic growth is influenced by increasing concentrations of metabolites or decreasing glucose concentrations in the culture media, the glucose uptake and growth of explants cultured with or without a change of serum or glucose supplement was examined. In experiments in which 10-5-day explants were cultured for 48 h the rates of glucose uptake and the embryonic size and protein content at the end of the culture were significantly impaired in the absence of either added glucose or a change of serum after 24 h of culture. There was, however, no difference between the explants cultured in media with glucose supplements and those given a change of serum, indicating that lack of glucose, but not high concentrations of metabolites, had an inhibitory effect on the development of 10-5-day explants after 24 h in culture. Clough & Whittingham (1983) have demonstrated that lactate can have a deleterious effect on embryonic growth in cultured mouse embryos but in their study the lactate concentrations were considerably higher than the maximum concentrations assayed in the culture media in the present study. In the subsequent experiment, 9-5-day explants were cultured for 48 h with or without periodic addition of glucose to the culture media to establish the dependence of 9-5-day explants on the concentration of glucose in the culture media. No differences were observed in the embryonic development within the two groups indicating that during a 48 h culture period the development of 9-5-day explants was not limited by glucose availability.

The gassing regimes used during the cultures were those found by New et al. (1976) to support optimum embryonic growth. Embryos of 9-5 to 10-5 days were cultured under a gas phase containing 5% oxygen in 5% carbon dioxide and nitrogen, the concentration of the oxygen was increased to 20% for embryos of 10-5 days and to 40% after 11-25 days. Other gassing regimes were not tested in this study because they had already been shown to have a deleterious effect on embryonic development.

Some differences were apparent between the cultures in each series of experiments. In experiment 2 there appeared to be a decrease in the rate of glucose uptake by explants between 42 and 48 h which was not apparent in experiment 1. Much of the difference is indeed an apparent difference because the 42 h assay was not initiated until the start of experiment 2. Another contributory factor to the differences could be the differences in the glucose concentration in the sera at the start of the cultures. In spite of efforts to standardize the conditions under which the rats were maintained prior to being used for bleeding, the glucose concentration in the serum before the start of the culture varied from 9-4 to 7-3 mM. In experiment 2, the low initial glucose concentration (7-3 mM) resulted, after 42 h of culture with normal glucose uptake by the explant, in medium with glucose concentrations as low as 2 mM. At such low glucose concentrations embryonic glucose uptake begins to be retarded, as can be seen by comparison of the two groups of explants in experiment 2b.

The other major difference between experiments was in the incidence of heart beat and yolk sac circulation in the embryos on examination at the termination of the cultures. All the embryos in experiments 1 and 3 had strongly beating hearts and good yolk sac circulations but only 50% of the embryos in experiment 2 had such good blood circulation. Since the poor circulation was equally distributed between both groups of embryos it is unlikely that it was an effect of the media glucose concentration. The embryonic growth and differentiation was excellent, it seems improbable that the blood circulation was impaired during the culture period. Embryonic heart beat almost always stops, temporarily, when the embryos are first removed from the culture bottles and placed under the microscope for examination. Provided the embryos are maintained at about 37°C the heart beat usually resumes after a couple of minutes. The embryos in experiment 2 had been subjected to more frequent interference during the culture than those in experiments 1 and 3, with the bottles being removed from the Rotators for sample collection for periodic initial glucose assays as in experiment 1 but also for subsequent glucose supplements and further assays. Each time bottles were removed from the Rotator, air, at room temperature, with its very low carbon dioxide content, enters the system. These frequent periodic fluctuations in culture conditions could have increased the sensitivity of the embryos to trauma, such as occurs at the termination of the culture.

Since the culture system has been shown to support similar rates of embryonic growth and development to those occurring in vivo and it has also been shown that embryonic glucose uptake is usually consistent between cultures, a more detailed study of glucose
metabolism, embryonic growth and the growth of the extraembryonic membranes was undertaken.

Glucose uptake by 9-5-day explants was independent of its concentration in the medium, within the range of 2-5 to 9-4 mm, indicating that the transport mechanism is not simple diffusion, the rate of which is dependent on the concentration gradient. It would thus seem probable that glucose uptake is dependent on a carrier-mediated mode of transport as has been demonstrated in many adult tissues. The small, but significant, increase in glucose uptake per unit of yolk sac could reflect an increased number of carrier molecules either as a result of increased cell folding at the apical border forming a greater number, or longer, microvilli or an increased density of carrier molecules within the membrane.

*In vitro*, the ratio of glucose uptake to lactate secretion by 9-5-day explants indicates that, for the first 42 h of the culture period, the embryonic glucose utilization is primarily by the glycolytic pathway. There have been no equivalent studies of the embryonic metabolism *in vivo* but, until the formation of the chorioallantoic placenta, the embryonic tissue is extravascular and is very slow flowing (Everett, 1935). Dependence on the glycolytic pathway is a characteristic of rapidly dividing cells (Papaconstantinou, 1967) where the relative lack of complexity of the pathways may facilitate regulation of cell metabolism especially during cell division. The glycolytic pathway is metabolically less efficient than the Krebs cycle but it yields ATP molecules at a greater rate. Between 9-5 and 11-5 days rat embryos undergo a 40-fold increase in protein content (New, 1973); with such extremely high growth rates the rapid production of ATP may be essential. The inefficiency of the pathway is probably unimportant while the demands of the embryos are so small relative to the total maternal reserves of carbohydrate. (Assuming the average litter size of CFHB rats to be 15 embryos and the embryonic glucose uptake *in vivo* to be similar to that occurring *in vitro*, the total glucose uptake by the entire litter between 9-5 and 10-5 days would be only 6 mg.) The embryos begin to use the more efficient pathways well before they become a significant drain on maternal resources. A reduction in the proportion of glucose converted to lactate occurred after 42 h in culture at which stage the rate of glucose uptake had increased to 1 mg per embryo per day. By the equivalent stage *in vivo* the chorioallantoic placenta is functional and supplied by the circulating maternal blood.

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**References**


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