Binding of Iron by Rat Placental Tissues

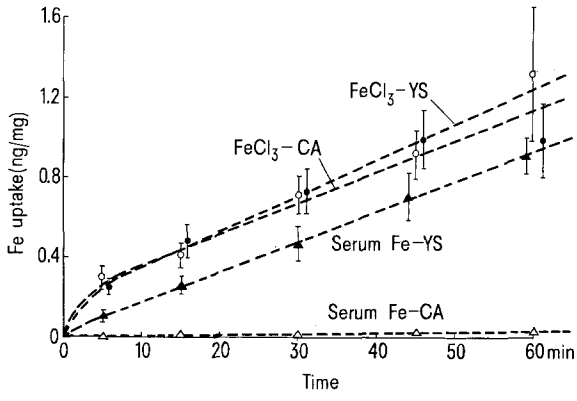
Several studies provide evidence that the rat chorioallantoic placenta is the main or exclusive route for iron movement to the fetus[1,2], while other studies suggest that the yolk sac placenta has a minor transmission role[3–5]. GARRETT et al.[6] obtained results which indicate that the yolk sac participates significantly in transmitting iron to the fetus on gestation day 16, but not on days 14, 18 and 20. GLASSER et al.[2] concluded that the placenta acts as an independent compartment between the fetus and the maternal plasma, and that placental accumulation of iron takes place when the fetus is removed. The object of the present study was to compare the iron-binding properties of chorioallantoic and yolk sac tissues on day 16 of pregnancy. Data were obtained in in vitro experiments. Iron was available in 2 forms, $FeCl_3$ and iron bound to transferrin in serum. Also the effects of metabolic inhibiting and proteolytic agents on iron-binding were observed.

Gravid Sprague Dawley rats of the Holtzman strain were the experimental animals. The sperm-positive date supplied (Holtzman Co., Madison, Wis.) was designated day one of pregnancy. The experiment was conducted on day 16. Surgical opening of the abdomen was made under pentobarbital anesthesia. The chorioallantois and yolk sac of each fetal-placental unit were detached and separated. The chorioallantois was sliced into 10 parts but the yolk sac was left intact. Each sliced or intact structure was placed into an ice-cold flask which contained modified Ringer-*Tris* solution (NaCl, 130 m*M*; KCl, 3 m*M*; $MgCl_2$, 1 m*M*; $CaCl_2$, 1 m*M*; *Tris* [2-amino-2-(hydroxymethyl)-1,3-propanediol], 19 m*M*; and glucose 0.02%). The total volume in each flask was 10 ml of which 1 ml was added as a solution of $FeCl_3$ or as serum from female, non-pregnant rats. The salt was added to supply 2.5 μg of iron, the quantity assayed in 1 ml of rat serum[7]. Then 2 μCi of $^{59}FeCl_3$ (Specific activity 16 μCi/μg; New England Nuclear) were added which resulted in a final 4.5 μ*M* concentration of iron in each flask.

The serum stood 1 h at 23°C for complete binding of the ^{59}Fe to transferrin which was confirmed by gel column chromatography. The pH was adjusted to 7.2–7.4. The pH and concentration of iron were within ranges that have been shown to yield completely soluble hydrolyzed products[8,9]. The flask preparations were agitated in a rotary shaker water bath (50 rev/min) at 37°C. After incubation the tissue was washed 5 times in the buffered salt solution to reach constant radioactive count. ^{59}Fe was counted in a NaI (T1) crystal well-type scintillation counter (Nuclear-Chicago). Data are expressed on a wet tissue weight basis. The metabolic inhibiting and proteolytic agents and their flask concentrations are presented in the Table.

The mean weights of the rat chorioallantoic and yolk sac organs were 336 ± 13 mg and 33 ± 1 mg respectively. Thus on day 16 of pregnancy the rat chorioallantois was about 10 times larger than the yolk sac and nearly the weight of the fetus, 343 ± 10 mg.

Uptake data are plotted in the Figure. Tissues from both placental organs bound a greater quantity of iron from $FeCl_3$ than from serum. The curves show near linear dependence with time. The serum-yolk sac curve



Uptake of iron by chorioallantoic (CA) and yolk sac (YS) tissues from iron (4.5 μ*M*) available as $FeCl_3$ or iron-transferrin in rat serum. The mean values ± SEM represent 10 to 20 paired organs (5 to 10 for each simultaneous time point).

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In vitro effects of chemical agents on iron uptake by the chorioallantois (CA) and yolk sac (YS) from the 16 days pregnant rat

Treatment	Number of organs CA	YS	Iron source[a]	Iron (pg/mg wet tissue) uptake in 1 h CA	YS
Control	10	11	$FeCl_3$	980 ± 210[b]	1710 ± 130
Sodium arsenite (5×10^{-3} *M*)	7	5	$FeCl_3$	1060 ± 120	1380 ± 140
Control	10	10	Serum[c]	31 ± 6	876 ± 132
Sodium arsenite (5×10^{-3} *M*)	6	6	Serum	43 ± 5	48 ± 18
Ethacrynic acid (1×10^{-4} *M*)	12	10	Serum	28 ± 5	474 ± 54
Rotenone (1×10^{-4} *M*)	6	5	Serum	27 ± 5	158 ± 54
Trypsin (0.01%)	6	5	Serum	19 ± 3	306 ± 51
Chymotrypsin (0.01%)	6	6	Serum	24 ± 3	621 ± 135
Papain (0.01%)	6	6	Serum	22 ± 3	204 ± 142

[a] 2.5 μg iron available for uptake. [b] SEM. [c] Non-pregnant rat serum.

shows that the yolk sac bound iron from serum nearly as well as from $FeCl_3$. The bottom curve in the Figure shows that chorioallantoic tissue had low capacity to bind iron from serum. In 1 h yolk sac tissue had bound 0.90 ng/mg iron while chorioallantoic tissue had bound only 0.03 ng/mg. This discrimination did not appear between the 2 placental tissues when iron was taken up from $FeCl_3$.

The effects of metabolic inhibitors on in vitro iron binding from rat serum by chorioallantoic and yolk sac tissues are shown in the Table. In one set of experiments $FeCl_3$ was substituted for serum. Yolk sac binding was reduced by sodium arsenite, rotenone and ethacrynic acid 95% ($P < 0.001$) 82% ($P < 0.001$) and 46% ($P < 0.001$) respectively. Rotenone and ethacrynic acid did not affect binding significantly in chorioallantoic tissue and sodium arsenite produced an unexpected 39% increase ($P < 0.01$). Ethacrynic acid and rotenone were dissolved in 0.4% ethyl alcohol. This concentration of alcohol did not significantly modify control values. Yolk sac iron binding from $FeCl_3$ decreased somewhat (19%) with sodium arsenite, but chorioallantoic binding was relatively unaffected.

The effects of proteolytic enzymes on in vitro iron binding from serum are shown also in the Table. Trypsin reduced binding in chorioallantoic and yolk sac tissues 39% ($P < 0.01$) and 65% ($P < 0.001$), and papain reduced binding 29% ($P < 0.025$) and 77% ($P < 0.001$), respectively. Chymotrypsin was the least effective, reducing binding in chorioallantoic and yolk sac tissues 23% ($P < 0.05$) and 29% ($P < 0.025$). It is not likely that the enzymes produced their effects through proteolysis of transferrin since AZARI and FEENY[10] have shown that transferrin is resistant to trypsin and chymotrypsin. Although iron and iron-transferrin have been reported to chelate with several compounds[11], no work has shown that iron-transferrin chelates with metabolic inhibiting or proteolytic agents. The responses to these agents by the yolk sac resembles the process which has been described for the reticulocyte by JANDL et al[12]. These have been used as arguments for cellular reception of iron from irontransferrin as being a process requiring metabolic energy and a protein receptor.

Previous in vivo experiments indicate that the rat yolk sac on days 14, 16, 18 and 20 has a relatively short-span capability of transmitting iron to the fetus (42% of intracardiac injected iron on day 16 only)[6]. There was no appreciable storage of iron either in the yolk sac or chorioallantois on those days. The present in vitro experiments demonstrate that yolk sac tissue has a high binding affinity for iron on day 16. This may be related to the apparent ability of the organ to transfer iron to the fetus on that day. The binding seems to be metabolically sensitive and is decreased by proteolytic enzymes, indicating the possibility of a receptor relationship.

Chorioallantoic tissue on day 16 has a low binding affinity for iron from serum. The binding is metabolically insensitive and is only mildly affected by proteolytic enzymes. Nevertheless, the chorioallantois, as previously shown on day 16, still apparently conveys the major portion of iron to the fetus (58%). At this time the physical size or bulk of the chorioallantois is about 10 times greater than the yolk sac. These results are made more interesting in light of the recent suggestion of MANSOUR et al.[13] that an iron transport system matures in the rat placental cell membrane between days 14 and 17 and becomes efficient by day 20.

Zusammenfassung. Nachweis, dass das Eisenbindungsvermögen der Dottersackplacenta bedeutend grösser ist als bei der chorioallantoiden Placenta und dass diese auch unter der Einwirkung von den Stoffwechsel beeinflussenden Stoffen ein verschiedenes Verhalten zeigt.

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Änderungen der Zellzyklusparameter chinesischer Hamsterfibroblasten unter chronischer Hypoxie, gemessen am Impulszytophotometer

Unter hypoxischen Bedingungen treten Änderungen im Proliferationsverhalten chinesischer Hamsterfibroblasten in vitro auf. Autoradiographische Untersuchungen der Flussraten von einer Zyklusphase zur anderen ergaben, dass auch die Verteilung der Zellen auf die einzelnen Zyklusphasen verschoben wird. Mit Hilfe der Impulszytophotometrie kann die Verteilung der Zellpopulation auf die verschiedenen Zellzyklusphasen nach zunehmenden Zeiten der Hypoxie gemessen werden.

Material und Methode. Chinesische Hamsterzellen vom Stamm B-14 wurden als Monolayer in Glas-Vierkantflaschen (Schott) kultiviert. Das Nährmedium bestand aus Eagle's MEM und 10% Kälberserum.

Zur Herstellung der hypoxischen Bedingungen wurden die Zellkulturen kontinuierlich mit einem wasserdampfgesättigten Stickstoff-Kohlendioxid-Gasgemisch begast. Zur Messung des DNS-Gehaltes der Zellen im Impulszytophotometer wurden die Zellen zunächst vom Boden der Kulturflaschen durch Trypsineinwirkung abgelöst, abzentrifugiert und mit 0,9%iger NaCl-Lösung gewaschen (Zentrifugation bei 200 g für 5 min). Ca 10^6 bis 10^8 Zellen wurden dann durch Eintropfen in 20 ml absoluten Alkohols fixiert. Dabei wurde die Probe zur Vermeidung von Zellverklumpungen apparativ geschüttelt. Nach 20 min wurden die Zellen durch Zentrifugation vom Alkohol getrennt und wieder mit physiologischer Kochsalzlösung gewaschen. Anschliessend erfolgte eine Resuspension und 1stündige Inkubation der Zellen in 10 ml einer 0,1%igen RNase-Lösung (50 E/mg, DNasefrei) bei 37 °C im Wasserbad. Nach erneuter Waschung der Zellen mit physiologischer Kochsalzlösung wurden die Zellen in 10 ml einer 0,001%igen gepufferten Ethidiumbromidlösung gefärbt.