Placental Transfer of Iron in Swine as a Function of Gestation Age.* (29143)

FRED H. HOSKINS AND SAM L. HANSARD†
Louisiana Agricultural Experiment Station, Baton Rouge

Prevalent anemias in growing swine are of economic concern, and the understanding of basic iron behavior and utilization in the developing fetus is of physiological significance for therapy and anemia prevention.

Early reports have indicated that iron traverses the placental walls in rats(1), rabbits (2), and during the final stages of gestation in pregnant women(3). Employing fetal hemoglobin and milk iron levels as a criteria of measurement, Pond *et al*(4) reported no significant effects of stable iron injections during late pregnancy and early lactation in swine. However, studies by Hoskins and Hansard(5) have suggested that iron levels increase in the sheep fetus with progressive age increases, and observed Fe^{59} to traverse the placental wall at all stages of gestation.

The increased interest in baby pig anemias, and the relatively small amount of information on the physiological behavior of iron in swine prompted this study on the maternal-fetal absorption and utilization of iron in swine as a function of gestation age.

Materials and methods. The experimental animals, 12 Hampshire gilts selected from the farm herd, were hand-bred and randomly divided according to breeding date into 3 groups for sacrifice at 35, 70 and 105 days gestation. Three non-pregnant gilts served as controls. All animals were maintained on a conventional corn-soybean-oil-meal gestation ration, containing 463 ppm iron, throughout the study. Each gilt was placed in an individual metabolism unit(6) equipped for the quantitative separate collection of urine and feces, for an adjustment period previous to administration of a single intravenous tracer dose of Fe^{59} citrate. Following a 48-hour blood-balance study, animals were sacrificed and selected maternal and fetal organs and tissues taken for radio-iron and total iron analyses. Radio-iron was measured by conventional methods using a well type scintillation detector attached to a scaler unit. The method of Barkan and Walker(7) was used for plasma iron, and tissue and fecal iron were measured using a modification of the procedure described by Wong(8). Fetal and maternal blood hematocrits and hemoglobin were measured by the cyanomethemoglobin method(9).

Results. Blood iron measurements serve as clinical criteria for evaluation of the physiological changes associated with anemias, and were employed in this study to support the radio-iron data. Hemoglobin values for dams were not significantly affected by gestation and averaged 11.34 $\pm$ 2.49 g%, while the mean fetal hemoglobin was 6.97 $\pm$ 1.14 g%. Fetal hemoglobin levels at the third trimester, however, were somewhat lower than values reported in the literature for new-born pigs(4). The fact that these fetuses were sampled approximately 7 days prior to parturition may have accounted for these differences, since the greater part of fetal iron transferred occurred during the latter stages of pregnancy. Cell-pack volume was essentially constant throughout gestation, and averaged 40.9% for dams and 32.1% for fetuses, respectively.

Fetal iron accretion with age and growth. Average fetal weight, crown-rump length, radio-iron and total iron content, and specific activity measurements, as a function of gestation age, are tabulated for swine in Table I. Between trimester one and two, fetuses increased 8 times in length and 30 times in body weight, while fetal length doubled and body weight increased 4-fold between the second and third trimesters.

Total iron concentration values decreased significantly with each progressive stage of pregnancy, and accumulative total iron con-

* This paper is published with the approval of the Director, Louisiana Agri. Exp. Station, Baton Rouge.
† Supported in part by National Science Foundation grant.

TABLE I. Average Weight, Length, and Radio-iron, Total Iron and Specific Activity of Whole Fetuses as a Function of Gestation Age in Swine.

Length (cm)	Wt (g)	No. fetuses	% dose g[1]	% dose Total	Iron (mg) g	Iron (mg) Total	S.A.[2]
				35 Days Gestation			
1.7	6.5	12	27.58 ± 6.9	.018 ± .007	.028 ± .018	.18 ± .04	1052 ± 394
				70 Days Gestation			
14.9	193.0	25	6.68 ± .9‡	.129 ± .025*	.021 ± .002†	4.25 ± 1.16‡	304 ± 55‡
				105 Days Gestation			
25.6	828.0	26	6.95 ± 2.60‡	.575 ± .028*	.018 ± .001†	14.90 ± 3.38‡	321 ± 85

[1] % injected iron-59 dose per gram, fresh weight, $\times 10^{-4}$, corrected to absorbed iron-59 (dam).
[2] Specific activity (SA) calculated as % dose Fe-59/g divided by mg iron/g, fresh weight, $\times 10^{-4}$.
* Denotes significance at P <10% level, † P <05%, ‡ P <01%, respectively.

tent showed highly significant increases with increased age. Whole body specific activity values decreased, significantly, between the first and second trimesters, and affirmed the rapid fetal incorporation and utilization of iron during the initial phases of gestation.

Placental complex, Fe-Fe^{59}. Iron accretion and partition in the placental complex (comprising placental membranes, fluids and fetuses) becomes as important physiologically in studies of this nature as iron partition (Table I) in the fetus itself. This is particularly true when considering dietary iron requirements and utilization, since these surrounding fluids and membranes must serve as the primary pool for fetal nutrition (Table II).

In non-pregnant control animals, the uterus contained only 0.3% of the absorbed Fe^{59} and 9.7 mg total iron. After 35 days gestation, these values for placenta increased sharply to 1.58% of the Fe^{59} and 31 mg elemental iron. During the second trimester, both radio-iron and total iron approximately doubled. Radio-iron in amniotic fluid remained essentially constant between the first and second trimesters, while total iron levels had increased by about 50%. Iron values for total fetuses, (standardized to litter of 8 pigs), are shown to increase markedly between trimesters one and two, with Fe^{59} increasing from 0.14% to 1.03%, and total iron from 1.5 mg to 34 mg. During the final trimester placental membranes of swine contained an average of 3.3% of the absorbed radio-iron after 48 hours. Total placental iron content was calculated to be 99 mg, therefore, while litters averaged 110 mg iron and amniotic fluid contained 62 mg elemental iron. Thus, iron crossed the placenta in the sow into the amniotic fluid against a concentration gradient, while this gradient favored passage of iron from the fluids to the litter, during the final trimester of pregnancy. Total fetal iron then comprised 43% of that present in the fetal complex at 105 days gestation.

Iron in fetal organs and tissues. The radio-iron, total iron and specific activity in selected fetal tissues and total organs, as a function of gestation age are shown in Table III.

Concentration values for Fe^{59} in fetal liver after 48 hours was highest during the first

TABLE II. Partition of Absorbed Iron-59* and Total Iron in Placental Complex† of Swine.

Tissue	0 Fe-59	0 mg	35 Fe-59	35 mg	70 Fe-59	70 mg	105 Fe-59	105 mg
	Days of gestation							
Placenta	.3‡	9.7‡	1.58	31.0	2.80	69.0	3.30	99
Amniotic fluid	—	—	.04	2.7	.03	4.3	.46	62
Litter	—	—	.14	1.5	1.03	34.0	4.60	119

* % absorbed dose, radio-iron.
† Includes placenta, amniotic fluid and swine fetuses.
‡ Uterus only.

TABL7 III. Radio-iron, Total Iron and Specific Activity of Selected Swine Fetal Organs and Tissues as a Function of Gestation Age.

Tissue	% iron-59 dose g[1]	% iron-59 dose Total[2]	Iron (mg/g)	S.A.[3]	SAd[4]/SAf
		35 Days Gestation			
Liver	127.0 ± 63.8	86 ± 23	.149 ± .001	859 ± 458	.78
		70 Days Gestation			
"	78.2 ± 32.5	460 ± 129	.221 ± .127	396 ± 107‡	2.89
Kidney	7.2 ± 4.7	7 ± 4	.025 ± .008	289 ± 166	2.24
Spleen	89.1 ± 73.4	77 ± 83	.397 ± .097	265 ± 182	1.51
Muscle	1.96 ± .3	—	.015 ± .007	155 ± 77	1.07
Femur, whole	10.93 ± 5.3	6 ± 4	.048 ± .027	243 ± 115	—
Rib, "	41.93 ± 39.6	—	.086 ± .058	599 ± 670	—
Sternum	18.89 ± 4.0	—	.229 ± .103	210 ± 126	17.83
		105 Days Gestation			
Liver	40.87 ± 6.92	1310 ± 267‡	.199 ± .027	213 ± 73†	5.15
Kidney	8.18 ± 4.6	27 ± 13	.043 ± .025	196 ± 67	2.97
Spleen	76.82 ± 43.8	108 ± 82	.146 ± .097	500 ± 231	.85
Muscle	1.68 ± .6	—	.014 ± .003	108 ± 35	2.29
Femur, whole	19.75 ± 3.9†	86 ± 18‡	.022 ± .003	877 ± 149	—
Rib, "	5.53 ± 1.3	—	.037 ± .004*	152 ± 42*	—
Sternum	16.97 ± 9.7	—	.045 ± .025	442 ± 309	11.76

[1] % dose iron-59 × 10^{-4}/g fresh weight, corrected to *absorbed* maternal iron-59.
[2] Calculated as % Iron-59 dose in total organs × 10^{-4} after 48 hr.
[3] Calculated as % dose iron-59/g divided by mg iron/g, fresh weight × 10^{-4}.
[4] Refers to ratio of tissue specific activity (S.A.) in dam to that of fetal tissues.
* Denotes significance at P <10% level, † P <05%, ‡ P <01%, respectively.

trimester of pregnancy and decreased progressively with increased age. Radio-iron content of the total liver, however, increased 5-fold between the first and second trimesters and, significantly, 3-fold between the second and third trimesters. This reflected in part the large increases in organ weight occurring with increased age. Total iron concentration values, however, showed no significant difference between the first and second trimesters, and remained essentially constant throughout the final stage of gestation. Specific activity values, therefore, decreased significantly and progressively throughout pregnancy, with the sharpest decrease observed between trimesters one and two.

Fetal age did not appear to influence Fe^{59} concentration values in either the spleen or kidney; however, radioactivity in total organs increased with age and weight. The significant decrease in iron concentration noted in the spleen between the second and third trimesters may have been a reflection of this organ's early iron contribution to hematopoiesis.

Concentration of Fe^{59} in the whole femurs increased 2 and 3 times during the second and third trimesters, respectively, while accumulative radio-iron content increased 14 times. Total iron concentration, however, appeared to be lower during this period and was reflected in the elevated specific activity values and relative iron turnover rate with advanced fetal age. Iron values for fetal ribs followed essentially the same pattern, except that total iron concentration decreased significantly during the last trimester, and specific activity values indicated a dilution by other minerals being accreted during this period. Fetal sternal Fe^{59} concentration showed no significant change during the last 2 trimesters of pregnancy, while stable iron concentration decreased significantly during the final stages of gestation.

The relative stability of iron and Fe^{59} values in muscle at the various periods of gestation suggested this tissue as a good reference for such studies.

Maternal-fetal specific activity ratios. These concentration ratio values, reflecting relative iron turnover rates for selected tissues and organs at the 3 periods of gestation, are tabulated in Table III. The fact that liver and muscle values increased somewhat with

TABLE IV. Summary of Total Iron in Swine Litters and Organs* as a Function of Gestation Age.

	Days, gestation					
	35		70		105	
Tissue	mg	%	mg	%	mg	%
Whole fetus (8)	1.5	100.0	34.0	100.0	119.0	100.0
Blood†	—	—	2.3	6.7	13.1	10.9
Liver	.7	48	10.4	30.5	50.9	42.8
Spleen	—	—	2.6	7.6	1.5	1.2
Kidney	—	—	.3	.9	.9	.8
Femur	—	—	.3	.9	.7	.6

* Values expressed as total mg iron per standardized litter of 8 pigs.
† Calculated from Hb values × 0.34, assuming 10% fetal total blood volume.

advancing pregnancy, while values for spleen and sternum decreased would indicate the increasing importance of the fetal liver to hematopoiesis. These data further show spleen and sternal red bone marrow contribution to be highest during the second trimester.

Total iron in litters and fetal organs. Total iron values for swine litters and the partition of iron in selected fetal organs as a function of gestation age are summarized in Table IV. The large proportion of total fetal iron contributed by the liver at 35 days gestation was of particular interest. This percentage decreased somewhat during the second and third trimester of pregnancy, but demonstrated the storage-function of this organ throughout gestation. Whole blood appeared to be the metabolic pool making the second highest contribution to litter iron values, with this importance magnified during the final trimester. Fetal spleen, kidney and femur, in that order, followed in relative contribution to total litter iron values in the developing swine fetus.

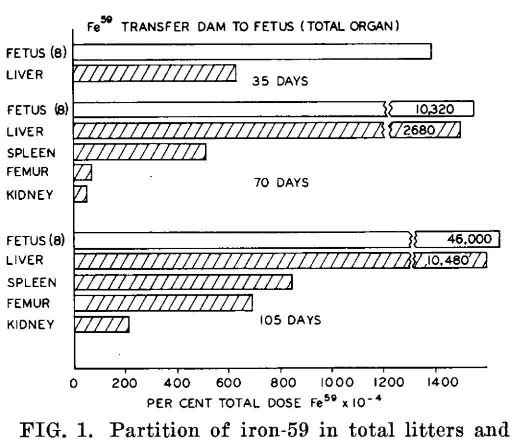

FIG. 1. Partition of iron-59 in total litters and organs of swine at 3 trimesters of pregnancy.

Iron-59 in swine litters and fetal organs. Fig. 1 shows total *absorbed* Fe^{59} in developing litters and relative partition after 48 hours in fetal organs. These values illustrate total radio-iron transferred from dam to swine litters (standardized to 8 pigs/litter) at the 3 periods of gestation, 48 hours after intravenous administration. The relative contribution of the liver to total fetal Fe^{59} traversing the placenta at 35 days is pointed out, and signifies the early contribution of this organ to hematopoietic function. The corresponding increases with advanced pregnancy observed for other organs would suggest initial liver storage to be at least partially distributed to these organs as needed. The spleen, during the second trimester and the spleen and femur at 105 days, further reflect contribution magnitude of these tissues to hematopoiesis in the developing fetus.

Discussion. Data presented here not only demonstrate the placental transfer and fetal tissue distribution of iron, but reflect a progressive pattern for hematopoietic activity in the prenatal pig. Fetal liver, spleen and red bone marrow showed increased iron storage throughout gestation. Hematopoietic activity and iron accumulation in the liver appeared at about 35 days gestation, while Fe^{59} activity in the spleen did not become significant until 70 days. Increased hematopoiesis in femur, rib and sternum occurred during the second and third trimesters and in that order, were indicative of the initiation of red bone marrow function in hematopoiesis that was reflected in total blood Fe^{59} values.

The rate and quantity of placental iron transport in swine was observed to be somewhat less than that reported for sheep(5). This could be explained by the differences in physical complexity of the 2 placentae. If placental transfer is governed partly by membrane complexity, then more iron would be expected to traverse the ovine than the porcine placenta, as reported.

Values in Table III show that a standardized litter of 8 pigs contained 119 mg iron at 105 days gestation. Since a total of 280 mg iron was accreted in the total fetal complex during the 105 day period, iron transfer to the total fetal complex per day was calculated as 2.67 mg. Therefore, if available iron was transferred into the placental complex as needed the added daily iron requirement of pregnancy in swine was 2.67 mg. Assuming maternal dietary absorption to be 10%, the sow would need to consume an additional 26.7 mg iron per day to meet these demands for fetal growth and development. The ration utilized in this study contained 463 ppm iron. From these figures it can be calculated, therefore, that an additional 57.6 g feed per day would be required to supply the additional 2.67 mg iron for the developing litter in a pregnant sow.

Summary. Results of radio-chemical studies with 9 pregnant and 3 control gilts dosed intravenously with a single tracer injection of Fe^{59} citrate, and sacrificed after 48 hours, indicated Fe^{59} values to increase 7-fold between 35 and 70, and 5-fold from 70 to 105 days gestation, respectively. Total fetal iron content increased from 0.18 mg at 35 days to 4.3 and 14.9 mg at 70 and 105 days, respectively. Fetal liver and spleen exhibited greatest contribution to hematopoiesis during early gestation, while these organs and red bone marrow became increasingly important as pregnancy advanced. Results of this study indicated that iron is transferred across the placental membranes of swine at each trimester, with quantity and rate increasing progressively with each stage of gestation.

Appreciation is expressed to B. Patterson, B. Cowan and Ann Gaydos for technical assistance.

1. Brunschwig, A. E., *Anat. Rec.*, 1927, v34, 237.
2. Bothwell, T. H., Pribilla, W. F., Mebust, W., Finch, C. A., *Am. J. Physiol.*, 1958, v193, 615.
3. Pommerenke, W. T., Hahn, P. F., Bale, W. F., Balfour, W. M., *ibid.*, 1942, v137, 164.
4. Pond, W. G., Lowery, R. S., Maner, J. H., Loosli, J. K., *J. Animal Sci.*, 1961, v20, 747.
5. Hoskins, F. H., Hansard, S. L., *J. Nutrition*, 1964, v83, 1.
6. Hansard, S. L., Plumlee, M. P., Hobbs, C. S., Comar, C. L., *J. Animal Sci.*, 1951, v10, 88.
7. Barkan, G., Walker, B. S., *J. Biol. Chem.*, 1940, v135, 37.
8. Wong, S. Y., *ibid.*, 1928, v77, 409.
9. Crosby, W. H., Munn, J. L., Furth, F. W., *U. S. Armed Forces Med. J.*, 1954, v5, 693.

Received January 31, 1964. P.S.E.B.M., 1964, v116.

Ethacrynic Acid: A Drug Which Abolishes the Concentrating Ability of the Kidney.* (29144)

K. MacGaffey,† L. A. LeZotte, Jr.,† J. H. Snyder, E. W. Moore and H. Jick
(Introduced by T. C. Chalmers)

Clinical Pharmacology and Renal and Electrolyte Divisions of the Medical Service, Lemuel Shattuck Hospital, Department of Public Health, Commonwealth of Massachusetts, and Department of Medicine, Tufts University School of Medicine

Numerous studies describing the effect of diuretics on the concentrating and diluting operations of the kidney have been reported (1-4). Such studies have led to information regarding localization of action of various diuretics in the tubule. Early, Kahn and

* This investigation was supported by grants from U. S. Public Health Service and by gifts from Merck, Sharp and Dohme Co.

† Work done during tenure of Clinical Pharmacology Traineeships, awarded to Tufts Univ. by Nat. Heart Inst.