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IRON METABOLISM IN HUMAN PREGNANCY AS STUDIED WITH THE RADIOACTIVE ISOTOPE, Fe^{59}*

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IN 1942 the results of a brief series of tagged iron absorption studies in pregnancy were reported.[1] It was found that a pregnant woman absorbed two to ten times the amount usually taken up by nonpregnant individuals. The value of the study was somewhat limited by the brevity of the series. The scarcity of the radioactive isotope of iron at that time precluded the possibility of maintaining standard dosage levels of feeding and therefore made interpretation of the results somewhat difficult. In the same year studies of the iron isotope distribution in fetal tissues and some observations as to the rate of transfer of iron from the maternal to the fetal organism were reported.[2]

In 1947 we reported preliminary observations in a study designed to determine the effects of some factors on the absorption of iron by the pregnant woman.[3] The further analysis of these data is now possible and constitutes the basis of this report.

In all, 819 pregnant women were given tagged iron. The data presented here are restricted to 466 women who returned for completion of the study and the processed blood samples from whom met radiochemical criteria of

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purity, including reproducibility of duplicate counts of radioactivity on two or more separate dates. Evidence that the 466 cases were representative of the total group of patients attending the Vanderbilt Prenatal Clinic is afforded by the fact that there was good agreement between the study group and total clinic patients with respect to distribution of attributes such as age, parity, number of weeks of gestation at the first clinic visit, outcome of pregnancy, laboratory values, and recorded dietary intakes.

Methods

The radioactive iron used was obtained by d,p bombardment of iron in the cyclotron, and therefore, was essentially the Fe^{59} isotope. Its purification has been described elsewhere.[4] From 200,000 to 1,000,000 countable counts per minute were administered. In terms of present-day concepts of safe radiation limits the amounts of activity used are well within limits of tolerance.* An additional assurance comes from the fact that the higher levels of 100-200 c.p.m. per milliliter of red cells obtained were only about one-tenth that used by others for studies of blood preservation and which have been tolerated with no demonstrable ill effects.[5] Nevertheless, it is not to be suggested that any radioactive tracer studies on human subjects are to be embarked upon lightly without due consideration and care for safe practices.

An unselected group of white women admitted to the Obstetrics Out-Patient Clinic of Vanderbilt University Hospital were fed single doses of iron tagged with the radioactive isotope (Fe^{59}) on their second prenatal clinic visit prior to any administration of therapeutic iron. The week of gestation during which this visit was made varied from patient to patient. Most of the subjects had uncomplicated courses, were nonanemic, and would be considered healthy pregnant women. The iron was stored as ferric chloride until immediately before administration, at which time an excess of ascorbic acid (about 4 mg. per mg. of iron fed) was added in order to reduce the iron to ferrous form. The reduced solution was given by mouth midway between meals. Dosage levels

*The question arises as to the amount of radiation delivered to the maternal organism following administration of these tracer doses of Fe^{59}. A high counting sample contained approximately 200 counts per minute per milliliter. The emcigency of our thin mica window counter for the beta particles from Fe^{59} is about 25 per cent. Thus the blood contained about 4×10^{-4} microcuries per milliliter. If the mass of absorbing tissue is large in linear dimensions compared with the maximum range of the beta particles the total dose in equivalent roentgens delivered by the complete decay of the isotope will be 500 C where C is the isotope concentration in microcuries per gram. Thus, if all of the activity were concentrated in large vessels the blood would receive 0.2 equivalent roentgens. Actually the radiation delivered to the adjacent tissues is of importance. A lower limit for this is obtained if the activity is assumed to be uniformly distributed throughout the body. Under these conditions if the blood volume is 10 per cent of the body weight the tissues would receive 0.02 equivalent roentgens. The actual figure should be somewhat larger than this but not more than ten times as great.

It may be argued that the total activity ingested will not necessarily appear in the circulating blood and that a portion will be located in higher concentration in tissues such as the liver or bone marrow. If the blood volume were 5 liters and only half of the activity were in circulation, then, the total iron absorbed would be 4 microcuries. If at any one time all of this were temporarily resident in the liver an upper limit for the dose would be the value delivered by the complete decay of the isotope. In a typical liver of 1.5 kg. weight this would be 1.3 equivalent roentgens. If, instead, the activity were concentrated in the marrow, assuming a total marrow weight of two-thirds that of the liver, the corresponding figure would be 2.0 equivalent roentgens. This is obviously an improbable occurrence.

As shown by Gibson[3] the radiation due to Fe^{59}, even under conditions used where much higher amounts of that isotope were employed, was considerably less in effect than that due to Fe^{55}. Since the d,p reaction on metallic iron in the cyclotron is notoriously inefficient for the production of Fe^{59} minimal quantities of this isotope were present in most of the iron used. The dosage is thus negligible in comparison with that from the Fe^{55}. Since the gamma radiation from Fe^{59} is diffusely and inefficiently absorbed, the dose will be considerably less than that for the beta particles computed for the case of uniform distribution in the body, i.e., less than 0.01 equivalent roentgens.

The dose rate will always be less than the initial rate. It is calculated by dividing the integrated dose by the mean life. Taking the largest figure for integrated dose, namely 2.0 roentgens, the maximum dose rate would be 0.02 equivalent roentgens per day. Since this is a liberal estimate of the upper limit we conclude that the dose rate would never exceed the presently accepted tolerance dose rate of 0.3 equivalent roentgens per week.

supplying 1.8, 2.0, 3.1, 3.2, 4.16, 4.7, 7.0, 9.0, 18.0, 39.0, and 120.0 mg. of *elemental iron* were used. Laboratory studies, including red cell count, hemoglobin, hematocrit, serum proteins, vitamin A, carotene, ascorbic acid, and others were done on the day the iron was given. Complete histories and dietary records were also obtained.

On the third visit, usually two weeks following administration of the tagged iron, duplicate blood samples were drawn for measurement. The blood was centrifuged in 15 ml. graduated cones for 35 minutes at 3,000 r.p.m. in a size 2 eight place head International centrifuge and the hematocrit was read. Plasma was discarded or used for other studies. The red cells were washed into 250 ml. beakers and dried. Dry ashing was carried out in a muffle furnace at 620° C. Walls of the beakers were washed down with dilute hydrochloric acid and samples again evaporated to dryness to eliminate excess acid. The mixed salts were dissolved in 5 or 6 drops of hydrochloric acid and transferred to electroplating cells with distilled water and the iron electroplated as described elsewhere.[4] The resultant electroplated samples were then measured for radioactivity by means of a thin mica window (2 mg. per square centimeter), end window type Geiger-Muller tube in conjunction with a counting rate meter. If the duplicate electroplated blood samples did not check within 5 per cent of each other the result was not included in the series.

Samples were counted twice at intervals of one week in order to check possible contamination from other isotopes being used in the laboratory. Blank samples of blood were run frequently, by the usual procedure, to check for iron isotope contamination. An aliquot of each iron sample fed was counted for an adequate period of time to note the rate of decay of the iron material. The relevant aliquot of sample fed was always counted on the same day as the blood iron sample.

The radioactivity per milliliter of red blood cells was calculated. The total circulating red cell mass was estimated as reported earlier,[1, 5] and the total circulating radioactive iron was calculated. It has been shown that, under normal conditions, all of the red cells are in active circulation, there being no considerable depot of sequestrated red cells.[6] It was necessary to make the assumption that all of the iron absorbed was utilized for red cell hemoglobin formation. We fully recognize the implications of this assumption in individuals not suffering from iron deficiency, but feel that for healthy individuals calculations based upon it are of value. The "percentage uptake" was estimated by dividing the total circulating radioactivity by the radioactivity in the sample fed the subject. The importance of the factor of utilization of absorbed iron is recognized.[7] Hence, "uptake" as here used must be construed to include both the factor of absorption and utilization. We have no evidence which indicates that utilization is impaired in normal pregnancy.

Experimental Observations

It was found very early in the study that, as expected, iron uptake was related to dosage level and to period of gestation. Hence a breakdown into subgroups according to both of these factors was necessary to evaluate the results. In Table I and Figs. 1 and 2 are summarized the most important findings of the study. Since some of the distributions of per cent uptake were quite skewed, the median per cent uptake, rather than the mean, was computed for each group. There was considerable individual variation in per cent uptake among the women within each group. Such variation has been pointed out before.[8] While variation of individual values about the medians should not be lost sight of, a study of the median percentage uptake shows the differences in the average behavior of the subjects for different dosages and different periods of gestation.

TABLE I. MEDIAN PERCENTAGE UPTAKE OF RADIOACTIVE IRON BY PREGNANT WOMEN ACCORDING TO DOSAGE AND PERIOD OF GESTATION

WEEKS OF GESTATION	DOSE 1.8-2.0 MG.		WEEKS OF GESTATION	DOSE 18 MG.		DOSE 39 MG.		DOSE 120 MG.	
	MEDIAN PER CENT UPTAKE	NUMBER OF CASES		MEDIAN PER CENT UPTAKE	NUMBER OF CASES	MEDIAN PER CENT UPTAKE	NUMBER OF CASES	MEDIAN PER CENT UPTAKE	NUMBER OF CASES
Under 15	11.0	13	Under 15	10.0	6	0.5	8	2.3	4
10-14	11.0	48							
15-19	17.0	37	15-24	10.2	15	12.5	31	0.5	8
20-24	32.4	53							
25-29	33.0	56	25 and over	20.0	10	16.5	13	8.0	21
30-34	40.3	55							
35 and over	41.0	23							

It is apparent that at a given dosage level the uptake of iron increases as gestation progresses from the tenth or fifteenth week up to the thirtieth or thirty-fifth week. Only a few cases were observed before the tenth or after the thirty-fifth week of gestation.

The percentage uptake of iron was greatest at dosage levels of 9 mg. or less and did not vary appreciably within the limits of 1.8 to 9.0 mg. The percentage uptake at the 18 mg. dose, however, was lower. Subsequent increases in dosage to 39 and 120 mg. were likewise accompanied by decreases in the per cent of the dose absorbed.

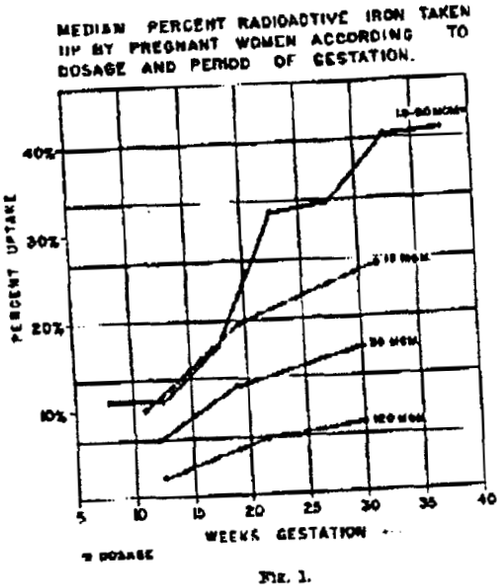

FIG. 1.

Fig. 2 shows the actual quantity of iron taken up (in milligrams) according to dosage and weeks of gestation. In this chart, dosages less than 9 mg. are further subdivided because the amount taken up varied as the dosage increased from 1.8 to 9.0 mg. It is again apparent that there is an increase in the quantity of iron absorbed as pregnancy progresses. It is further evident that, even during the period of maximum absorption, a six to sevenfold increase in the quantity of iron fed (from 18 to 120 mg.) results on the average in approximately a twofold increase in the amount of iron taken up. During the early part of pregnancy, this increase in quantity of iron absorbed is even less as the dose is raised above 9 mg. (Fig. 3).

It has been demonstrated[9] that some of the iron ingested by the pregnant woman crosses the placenta and is transmitted to the fetus. It is of interest, however, to explore the extent to which this process occurs and the influence upon it of such variables as dosage level and period of gestation. Accordingly, in 68 cases blood was taken at the time of delivery from the fetal umbilical cord and from the mother as nearly simultaneously as possible, and the amount of radioactive iron per milliliter of red blood cells was determined for each sample. On the basis of the estimated red cell mass of the infant, not including the

placenta, the total per cent of the dose given the mother which was present in the infant's circulation was calculated. The median per cent of the administered dose present in the infant's red cells is shown in Table II, according to dose and weeks of gestation when the dose was given. Parenthetically it may be noted that good agreement between the initial estimate of iron absorption by the mother and that obtained by the use of samples taken at parturition supports the interpretation of our findings in terms of absorption. Had any appreciable absorbed iron been stored and then used later in pregnancy for maternal hemopoiesis, the estimate of uptake made at the time of delivery should have been greater than that based upon the earlier sample. This did not occur.

TABLE II. MEDIAN PER CENT OF ADMINISTERED IRON PRESENT IN THE INFANT'S RED CELLS AT BIRTH ACCORDING TO DOSAGE AND PERIOD OF GESTATION WHEN IRON WAS GIVEN

	WEEKS OF GESTATION	MEDIAN PER CENT OF DOSE	NUMBER OF CASES
Doses 1.8-9.0 mg.	Under 20	1.5	11
	20-29	2.2	24
	30 and over	3.0	22
	Total	2.5	57
Doses 18, 39, 120 mg.	Total	0.9	11

At dosages of 9.0 mg. and under, the differences in per cent uptake of fetus at different periods of gestation are suggestive but not significant.

The difference in per cent uptake of fetus between low and high doses is significant (P = .02).

It appears that at the smaller dosage levels some 2 or 3 per cent of the administered iron on the average was found in the red cells of the infant at birth. At the higher dosage levels (18 to 120 mg.) the median percentage of the dose found in the infant's circulation was significantly lower, 0.9 per cent. The relationship between the percentage of radioactive iron found in the red cells of the newborn and the period of gestation when the iron was given cannot be determined with finality from our data because of the small number of cases. The group with dosages between 1.8 and 9.0 mg. was subdivided according to weeks of gestation at the time the iron was administered. There appears to be a slight increase in median percentage uptake of iron by the fetus from the earlier to the later weeks of gestation, but the difference is not statistically significant. A large series of similar observations is needed in order to establish the basic pattern of fetal iron uptake and utilization for hemopoiesis during different periods of pregnancy. However, in view of the proportional size of the fetus it would appear from these observations that the quantity of iron taken up by the fetus is relatively greater in early fetal life than at a later period. This interpretation is consistent with the earlier work of Pommerenke and associates[10] in which a study was made of the transmission of ingested iron to the fetus of

TABLE III. RATIO OF FETAL TO MATERNAL RADIOACTIVE IRON PER MILLILITER OF RED BLOOD CELLS ACCORDING TO DOSAGE AND PERIOD OF GESTATION WHEN IRON WAS GIVEN (MEDIAN VALUES)

	WEEKS OF GESTATION	MEDIAN RATIO	NUMBER OF CASES
Doses 1.8-9.0 mg.	Under 20	1.00	12
	20-29	1.08	22
	30 and over	1.22	17
	Total	1.12	51
Doses 18, 39, 120 mg	Total	0.86	10

different ages obtained during therapeutic abortions. In these studies the fetal red blood cells took on proportionately much more radioactive iron early in pregnancy than did the maternal red blood cells.

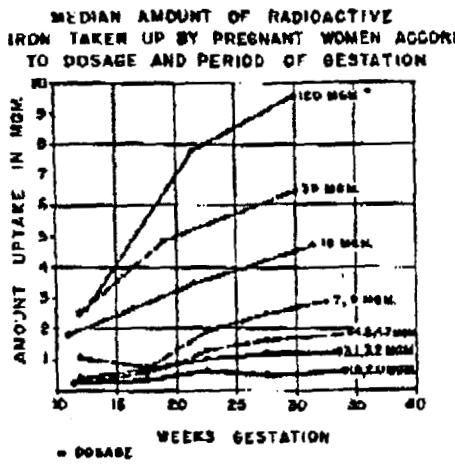

Fig. 2.

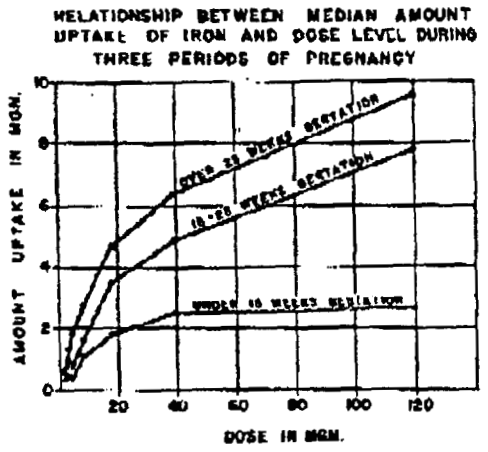

FIG. 3.

Table III presents the ration of fetal to maternal radioactive iron per milliliter of red blood cells by dose and time of gestation when the iron was administered. The median ratio was close to unity in all groups; moreover, in individual cases, 90 per cent of the ratios lay between 0.50 and 2.00. There seems to be a surprisingly similar distribution of radioactive iron between the fetal and maternal red blood cells. The median ratio was somewhat higher in the latter part of pregnancy, but the differences are not statistically significant so that again no definite conclusion can be reached.

The ratio of the estimated total percentage uptake of iron by the infant's blood to the total percentage uptake by the mother's was also computed. Median ratios of 0.07 to 0.10 were obtained, regardless of the time of gestation or the dosage level administered. In other words the blood of the infant at birth seemed to contain some 7 to 10 per cent as much radioactive iron as the mother absorbed and incorporated in her red cells.

A study was made of the effect of parity on iron uptake since it seemed reasonable to expect that frequently repeated pregnancies might result in the depletion of the iron reserves of the mother and this, in turn, might be reflected in augmented absorption. The data in Table IV show the median percentage uptake by parity and time when iron was given for patients receiving doses of 9 mg. or less. In this group, the mothers who received iron before twenty weeks of gestation did, in fact, exhibit an influence of parity upon uptake of iron. Before the twentieth week of gestation, the median per cent uptake was significantly higher in the higher parity groups. In the second half of pregnancy, however, parity did not influence the uptake. Results for patients receiving higher doses of iron (18 to 120 mg.) were consistent with those at the lower doses, but the number of cases is too small to permit the drawing of definite conclusions in the higher dosage group. These differences in iron uptake with parity are not the result of changes in body weight in association with parity. It is conceivable that they reflect lower iron stores in the multiparous women, and that the normally increased uptake late in pregnancy masks this finding during the latter half of gestation. There was no evidence in our series of a variation in the per cent uptake of iron by the fetus with parity of the mother, but again the number of cases is small.

TABLE IV. MEDIAN PER CENT UPTAKE OF RADIOACTIVE IRON AMONG PREGNANT WOMEN ACCORDING TO PARITY AND PERIOD OF GESTATION
(DOSAGES OF 9 MG. AND UNDER)

WEEKS OF GESTATION	PARITY					
	0		1-2		3 and over	
	MEDIAN PER CENT UPTAKE	NUMBER OF CASES	MEDIAN PER CENT UPTAKE	NUMBER OF CASES	MEDIAN PER CENT UPTAKE	NUMBER OF CASES
Under 20	10.5	49	13.5	41	19.3	35
20 and over	35.0	93	34.5	50	30.0	38

We have explored the possibility of a relationship between uptake of radioactive iron by the mother and numerous laboratory measurements which were made in an effort to appraise nutritional level. These have included hemoglobin, packed cell volume, erythrocyte count, total serum protein, serum albumin, vitamin A, carotene, and ascorbic acid levels in the serum, and urinary excretion of thiamine, riboflavin, and N-methylnicotinamide following oral test doses of each. No relationship has been found for any of these laboratory measurements except an unexplained association with serum ascorbic acid levels in one group. In the group of mothers at twenty weeks' gestation or less, it was found that those with a low per cent uptake of iron had high ascorbic acid levels. We feel that this association is fortuitous. This belief is supported by the fact that there was no similar association with estimated ascorbic acid intake.

Failure to demonstrate an association between hemoglobin level and percentage of iron absorbed is attributable to the fact that most of these patients were healthy, nonanemic individuals. If one were dealing with a group of pregnant women, a considerable portion of whom exhibited hypochromic anemia,

one would expect to find a relationship between hemoglobin level and iron uptake.

Efforts were made to correlate per cent of iron taken up with nutrient intake. Although the possible relationships were explored for a variety of nutrients, including total calories, protein, iron, calcium, phosphorus, vitamin A, vitamin C, thiamine, niacin, and riboflavin, no association was established.

Discussion

These data on the uptake of iron during pregnancy by a group of women, most of whom were normal, have some interesting implications concerning the administration of supplementary iron to the healthy pregnant woman. For this purpose, data on the actual amount of iron absorbed at each dose, rather than the percentage absorption, are pertinent. Thus the absorption of 85 per cent of a 2 mg. dose would result in less than one mg. of iron entering the circulation, whereas an uptake of 28 per cent of an 18 mg. dose would allow approximately 5 mg. to enter the blood stream. With an uptake of 8 per cent of 120 mg., approximately 10 mg. of iron would be taken into the circulation. This estimate coincides with the median per cent uptake of a 120 mg. dose administered during the latter portion of gestation. This is an especially interesting figure inasmuch as it corresponds to the maximum therapeutic dose of iron that is likely to be ingested at one time; namely, a dose of 0.325 Gm. of dessicated ferrous sulfate. This is somewhat larger than the dose of iron which would be ingested from the taking of approximately 5 grains (0.325 Gm.) of crystalline (hydrated) ferrous sulfate or 3 grains (0.195 Gm.) of dessicated ferrous sulfate, the quantity contained in some of the widely employed tablets. Such tablets contain approximately 65 mg. of elemental iron.

From Fig. 3, which depicts the median amount uptake of iron in milligrams plotted against the dosage administered during an early, middle, and later period in pregnancy, it is seen that only slight advantage is to be gained in terms of milligrams of iron absorbed and utilized by a threefold increase in dose (from 39 to 120 mg.) of iron during the period after twenty-five weeks of gestation. During early pregnancy relatively little iron was absorbed and utilized by the average patient at any of the dosage levels used. Unfortunately, the number of patients receiving the higher doses early in pregnancy was rather small (Table 1). A corollary to these considerations is that so-called "preventive" or "protective" iron therapy, even when intensive, may be a relatively inefficient procedure early in gestation. In the latter part of gestation, such protective therapy may be more efficient, but it would seem that single doses of 120 mg. of iron offer little advantage in terms of actual iron incorporated into the maternal red blood cells over considerably smaller doses.

Since only a single dose of iron was administered in these studies, it is probable that the figures arrived at are maximal, inasmuch as it has been shown that iron feeding leads to resistance or mucosal block to the absorption of iron[8, 9] and it may be that oft-repeated doses would be less efficiently absorbed than singly administered ones. If this concept holds for the pregnant woman, it would cast further doubt upon the usefulness of the administration of large doses of iron to the normal pregnant woman.

It is not clear what interpretation should be given to the relatively small amount (or possibly lack) of variation in uptake of the fetus with different periods of gestation, as measured by the amount of radioactive iron in the blood of the infant at birth. Whether studied in terms of percentage of total dose administered, percentage of the uptake of the mother, or ratio of amount of radioactive iron per milliliter of red blood cells of the infant to that of the mother, little variation was observed in the results for dosages administered at different periods of gestation.

Summary

1. Iron tagged with the radioactive isotope Fe^{59} was fed in single doses, ranging from 1.8 to 120 mg., to 466 women at various stages of pregnancy, and the uptake of iron determined.

2. As the dosage was increased from 9 to 18 mg. and beyond, the percentage of the total dose of iron found in the maternal red cells decreased, while the amount of iron taken up increased from the lowest to the highest dose.

3. As gestation progressed, the uptake of iron increased to the extent that at thirty weeks' gestation and over, three or more times as much iron was absorbed as during the period before the fifteenth week of gestation.

4. The therapeutic implications of these findings are discussed. The inefficiency of absorption of large doses of iron for normal pregnant women is noted.

5. The variation in uptake of the fetus with varying dosage does not appear to be greatly different from that of the mother. Relatively little variation in the amount of radioactive iron in the red cells of the infant at birth was observed for iron administered at different periods of gestation. The red blood cell mass of the newborn infant contained on the average about 10 per cent as much of the administered iron as that of the mother. The ratio of fetal to maternal radioactive iron per milliliter of red blood cells was not greatly different from one.

6. The relation between parity and iron uptake is discussed.

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