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Exogenous iron metabolism in pregnancy

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Exogenous Iron Metabolism in Pregnancy

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When women come to prenatal clinics or to private physicians to be followed during their pregnancies, the first procedures obtained in the examination are a hemoglobin and red blood cell counts. Then even before the results of these tests are returned, the patient is placed on oral iron therapy such as ferrous sulfate in the dose range of 100 mg. per day. The patient is then followed throughout her pregnancy with further hemoglobin values to determine the effectiveness of the medication or any preexisting anemia and to make sure that the patient does not become anemic during her pregnancy.

What is the reason for this concern with anemia in pregnancy? One reason for concern is that pregnancy is a normal physiologic process in the female of most species and is necessary for further propagation. Yet, for this important process, the female of the species has apparently not been made entirely adaptable without outside aid. This occurrence would be a very unusual circumstance. What is so unusual about the metabolism of iron in women during pregnancy that leads to iron deficiency anemia and what effect does this iron deficiency have on the fetus in these women who are iron deficient? Finally, what is the effect and fate of large amounts of iron given to these women? The answers to these questions are not all found in the current literature, part of which will be reviewed in this paper. Two of the reasons that these questions have not been answered is because of a lack of new studies on these women, and secondly, because of a lack of means for studying iron metabolism in these women. In the nonpregnant patient it is relatively easy

to study iron metabolism by the use of radioactive Fe^{59}; however, because of the unknown danger to the fetus from such small amounts of radioactivity administered to the mother, the use of Fe^{59} in this case is prohibited. Before this inhibition was placed on the use of Fe^{59} several studies were done with the radioactive isotope and these will be presented later. [1,2] Although much is known about iron metabolism in pregnancy, and the use of supplemental iron in pregnancy has become an accepted procedure, it has been shown that several questions have not been satisfactorily answered. In this paper, these questions will be defined by a brief review of the current knowledge of intermediary iron metabolism in pregnancy. Following this review a new research technique for following iron during its metabolism will be presented. This method is felt to offer great promise in continuing ferrokinetic studies in pregnancy where the use of Fe^{59} has stopped. That a tracer system is a valuable tool will be seen in the work to be presented which has been done with Fe^{59} to gain knowledge of iron metabolism in pregnancy.

It was not until the year 1925 that any effective work on the metabolism of iron in pregnancy could be done because prior to this time classical views predominated stating that the sole source of iron for the fetus was the maternal red blood cell. However, in 1925, it was found that the plasma could contain minimal amounts of iron.[3]

Iron Absorption From the Intestine

The metabolism of administered iron in pregnancy begins as it enters the gastrointestinal tract. The usual form for oral adminis-

Dose Weeks of Gestation	1.8-9.0 mg. % uptake	18 mg. % uptake	39 mg. % uptake	120 mg. % uptake
Under 15	11-17	10	6.5	2.2
15-24	17-32	19.5	12.5	6.5
25-40	33-41	26.0	16.6	8.0

(Figure 1)[1]

tration is ferrous sulfate. The iron is absorbed from the gut in the ferrous form bound to ferritin. Pregnant women can absorb from two to ten times the amount that is normally absorbed. In pregnancy the amount of iron absorbed is related directly to the dosage levels and to the period of gestation. By increasing the dose of orally administered iron, Hahn has shown that there is a decreased percentage of the iron absorbed as the dose is increased, but the actual amount of iron absorbed is greater.[1] Also, as the gestational age of the fetus increased the percentage of iron absorbed is increased. (Figure 1)[1]

There are also several other factors which influence the rate of uptake. The first is the degree of anemia. With more severe anemia more iron is absorbed. The rate of uptake is increased also as parity increases. Compared to primigravidas iron uptake up to 20 weeks gestation is greater in multiparous women. After this there is no difference in the uptake. However, there is no evidence that the fetal uptake of iron is increased under these circumstances. (Figure 2)[1]

Iron Transport

After the iron is absorbed through the mucosa it is bound to another protein molecule, transferrin. Iron is bound to transferrin in the ferric form. Once the iron is bound to transferrin it is in a

Parity	0	1-2	3 or more
Weeks of Gestation	% uptake	% uptake	% uptake
Under 20	10.5	13.5	19.5
Under 20	35.0	34.0	30.0

(Figure 2)[1]

form which is readily transportable to the various sites in the body for use. This is necessary since free, or unbound iron, is quite toxic.

The binding of the iron molecule to the protein transferrin in the plasma provides the basis for two of the clinical determinations which are done to determine the amount of iron deficiency or excess. These tests are the serum iron and iron binding capacity. The serum iron measures the actual iron content of the serum. In the non-pregnant female the normal value is 60-120 umg./100 cc.; however, in the pregnant female the normal value is increased and Holly considers the minimum normal value in these women to be 100 ugm./100 cc. with anything below this suggesting iron deficiency.[4] This value (from previous work done at University Hospital) appears to be high and as long as women are in the normal range for serum iron, pregnant or not, they should not be considered iron deficient if the other hematologic parameters are normal. It is important to realize that during pregnancy there is a diurnal variation in the serum iron. This is important only from the standpoint that if serial samplesare to be used for serum iron they must be drawn at the same time of day.

The second evaluation which can be made of the patient's serum with regard to iron status is the serum iron binding capacity. This measures the amount of unsaturated transferrin, i.e. the additional

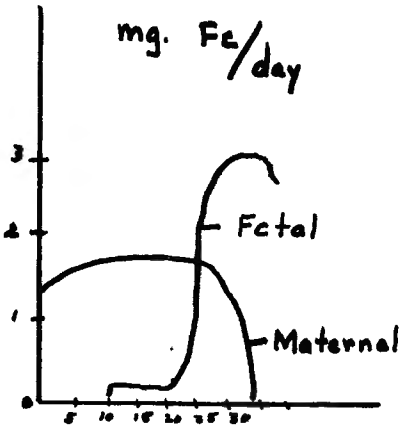
capacity of the body to bind more iron for circulation. This index is useful in that if one finds a low serum iron and high serum iron binding capacity, he can be more confident that he is dealing with a problem of iron deficiency. However, during pregnancy the serum iron binding capacity is greatly increased due to an increase in transferrin. Thus a high serum iron binding capacity would not be indicative of iron deficiency unless coupled with a low serum iron.

Iron Distribution in the Maternal-Fetal Complex

From the plasma the direction of iron metabolism assumes two major routes. The first of these is to the maternal marrow where the iron is used in the manufacture of the maternal red blood cells. In pregnancy there is an increased need for the iron at this site because of the increased red blood cell volume. Fortunately, concerning the marrow iron demand, the rate of red blood cell metabolism is not as great as would be expected from the plasma volume increase or as is seen in anemic states. This suggests that during pregnancy the body has some mechanism of preventing the marrow from reaching maximum red cell production, such as would be seen in treated iron deficiency anemia in the nonpregnant patient. Thus the theoretical demand for iron is decreased. Holly[4] has found that instead of the erythroid hyperplasia as would be expected in bone marrow specimens. There exists only a mild pancellular hyperplasia with the ratio of cell types remaining the same as that followed in the nonpregnant individual.

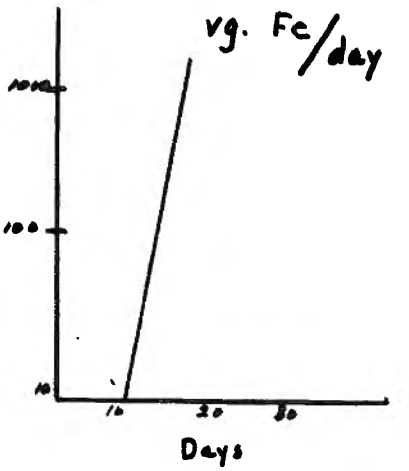
Placental Iron Metabolism

By the end of pregnancy approximately 90% of an administered dose of iron is found to be metabolized by the placenta.[5] Radio--



Days of Pregnancy (Rabbit)

(Figure 3)[6]



Transplacental Iron Transport (Rabbit)

(Figure 4)[6]

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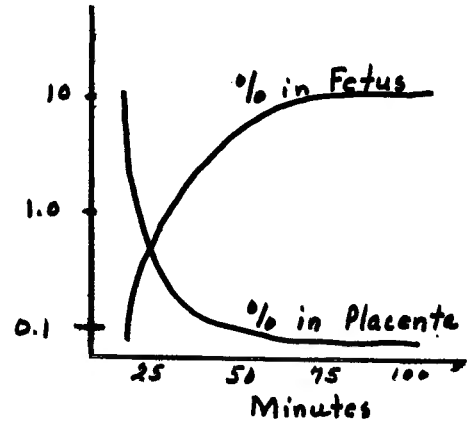
active iron injected into the mother during labor rapidly appears as iron in the fetal circulation not bound up in hemoglobin.[4] The question then arises, what is the nature of the placental transport of iron? It obviously must be more than simple diffusion of the iron across the placenta because the 90% uptake of iron by the fetus is a gradually increasing rate of uptake from conception. (Figures 3 & 4)[6]

As can be seen in figures 1 and 2, the iron is constantly moving towards the fetus despite a consistently higher fetal plasma iron and a higher saturation of fetal transferrin than the maternal transferrin. The first important study of placental transport was done by Pribilla, in 1958.[7] He noted that there was a competition for iron between tw o acceptor systems, the maternal marrow and fetal tissues. Yet in spite of this competition, the amount of iron delivered to the

RATIO: $\frac{\text{Placental activity}}{\text{Fetal Activity}}$



(Figure 5)[6]

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to the placenta by maternal transferrin is adequate for the needs of the fetus. The basic stimulation for this transfer is assumed to be the fetal need since the uptake of iron is proportional to the weight of the fetus and not that of the placenta.[1] When studying the placental metabolism Pribilla first attempted to prove that there was no accumulation of iron in a placental pool from which it was transferred to the fetus. He did this by taking term rabbits, injecting Fe^{59} intravenously, then taking samples from the placenta and fetus at various times following injection and determining the Fe^{59} content of both. The results of this experiment can be seen in Figure 5.[6]

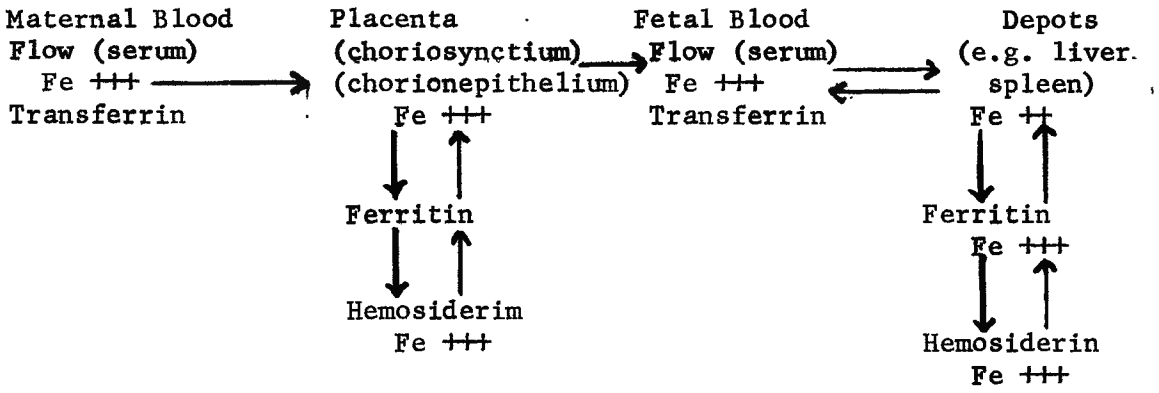
As can be seen from these results, there is no significant holdup of radioiron in the placenta. Transfer of iron from maternal plasma to placenta had a $T\frac{1}{2}$ of 15 minutes and uptake of iron by the fetus a $T\frac{1}{2}$ of 25 minutes, and the ratio of placenta to fetal activity dropped rapidly over 30 minutes from 12 to less than 0.1 and stabilized at the latter level.

The next question concerning placental metabolism which Pribilla answered was: is there an exchange of iron in a direction from the fetus to the mother, or is the transfer of iron unidirectional? To answer this question, Pribilla injected Fe^{59} intravenously into one of sibling rabbit fetuses while still in utero. He then waited one hour and sacrificed the animals. He found that in the sibling fetuses which had not received injections of Fe^{59} no radioactivity was found. Since as had been seen previously, 90% of any iron in the maternal circulation is rapidly taken up by the fetus, one could expect any iron which had been put into the maternal circulation to be found in the non-injected fetuses. "The absence of any evidence of uptake in this experiment can be taken as strong evidence that there was no feedback of iron from fetus to mother."[6]

Pribilla interpreted his experiments to show a close parallel between increased fetal size and increased amount of iron transfer, implying fine regulation of iron transfer by the fetus. Apparently this does not occur through greater pull from fetal transferrin, since this is saturated with iron at the time when fetal uptake is greatest. It also seems unlikely that the uterine blood flow or placental size accomplishes this adjustment since the increase in size of these structures does not coincide with increase in fetal needs. The increase in uptake by the fetus is to some extent influenced by the available iron in the maternal circulation.

Wohler[7], however, disagreed with the theories proposed by Pribilla. He attacked the theory that the placenta would allow free one-way diffusion of iron. To do this he injected 30 mg. of iron sorbitol intravenously into rabbits in the last stages of their

pregnancies. This gave a serum iron level in the mother of over 4000 ugm. per cent after five minutes, but a value of 165 ugm. per cent in the fetus. An elevated iron level was found in all organs of the mother, especially the kidney. However, the increase in the iron content of the fetal area of the placenta, as well as the fetal liver, kidney, lung, and heart showed that there is no true penetration of the placental barrier even in the presence of maximum serum iron values. In a second experiment, the time interval for getting blood samples was increased to 15 minutes. In this set of experiments the maternal serum iron level was found to be only slightly over 300 ugm. per cent, while the values in fetal plasma were over 200 ugm. per cent. Wohler assumes that these later values of increased fetal plasma iron are consistent with the normal iron absorption in the placenta. In summary, Wohler believes that his data indicate that ferritin formation in the placenta removes as much iron from the maternal plasma as the fetus requires. Thus, the ferritin of the placenta probably functions as a safeguard for procession of iron to the fetus. Theoretically this may lead to iron deficiency in the fetus whenever the placental iron supply becomes too small. This occurs only in chronic severe maternal iron deficiency. In cases of slight iron deficiency it is possible to demonstrate some increases in the ferritin-iron fraction and a marked increase in total organic iron which indicates a depot function for iron in the placenta. Wohler's concept of the intermediary metabolism of iron is seen in Figure 6.[8] Further evidence for synthesis of ferritin in the fetus is seen in work done by A. R. Rausen.[8] He has shown by starch electrophoresis of cord serum



(Figure 6)[8]

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that two types of transferrin are found, types C and D. Type C is found only in the fetus and therefore probably synthesized only on the fetal side of the placenta.

Both Pribilla and Wohler agree that the prime controller of iron transfer across the placenta is the need of the fetus. Other factors controlling the iron transfer are infection and steroids, especially progesterone which seems to decrease the placental transfer of iron.[9] M. Conti[10] has found that the corticosteroids and aldosterone increase the placental transfer of iron. He suggests that the placental transfer of iron based on these findings is an enzymatic process. This would add a third theory to those of Pribilla and Wohler on the intermediary metabolism of iron pregnancy. Further work on this theory needs to be done.

Assuming from what has been presented thus far that the placental transfer of iron is an active process rather than simple diffusion, is there any sacrifice made by the pregnant female concerning distribution of iron in her red cell mass as compared to the nonpregnant woman? That there is a difference in rats was shown by Hogburg and

Lindvall.[11] They injected 3 ml. of an iron dextrin complex containing 50 mg. Fe/ml. into pregnant and non-pregnant rats. This iron was labeled with Fe-59, 50 uc./ml. The results are seen in Figure 7.[12] As can be seen from this data, the increase in fetal iron occurs at the expense of the mother. Although it appears that the maternal red cells have a greater uptake of the iron initially this is most likely due to the increased red cell turnover and increased iron uptake by the marrow and as the length of time after injection is increased, the percent of iron in the fetal red cells is increased while that in the maternal cells is decreased.

Hogberg[12] has also shown that in the normal fetal-placental relationship that there is relatively little iron stored in the placenta as compared to that transferred through the placenta; however, he has also shown that without the fetus attached to the placenta it still has the ability to take up iron from maternal blood, thus supporting Wohler's[8] theory of active transfer of iron by the placenta. The results of Hogberg's work are seen in figure 8[11]. In looking further at figure 8 there seems to be little actual storage of iron in the placenta if the circulation is intact. Further proof that there is little storage in the maternal placenta is seen in the work of Davies[12] with Fe-59 in rabbits. At 13 days gestation he found that 97% of the total recovered radioactivity was present in the fetal placenta 7 minutes after intravenous administration of Fe-59 to the mother. At 18 days gestation this amount increased to nearly 100%. Also Davies showed that the ratio of fetal to placenta iron increases with an increase in gestational age. See figures 8, 9, and 10.

Quantity of Fe-59 in Serum in uc./ml.

Rats	3 hr.	6 hr.	1 day	2 days	3 days	4 days
Pregnant	0.58	0.54	0.30	0.23	0.19	0.13
Non-pregnant	0.17	0.12	0.12	0.10	0.09	----

Quantity of Fe-59 in 3 ml. of Packed Erythrocytes in uc.

Rats	3 hr.	6 hr.	1 day	2 days	3 days	4 days
Pregnant	0.21	0.56	0.99	4.07	4.59	6.08
Non-pregnant	0.23	0.19	1.12	2.62	2.97	----

(Figure 7)[12]

Davies feels that the reduced concentration of iron later in gestation seen in the yolk sac and placenta are accounted for by the increased production of fetal red blood cells. Also, he has noted that the embryo appears to be saturated with administered iron three hours after injection of Fe-59

As we have seen, the rate of uptake of iron by the placenta increases as the gestational age increases. In ewes it has been shown that the total radioiron in whole fetuses increases 14 times in the second trimester and four times in the third. Only part of this increase is due to an increase in weight on the part of the fetus, the remainder being due to increased placental transfer. It has been shown that as gestational age increases there is also a change of distribution of iron in the fetus. See figure 10. Once again there is evidence that the placental transfer or iron is an active process and not a process of passive diffusion.[13]

Quantity of Fe-59 in placentas without fetal circulation; placentas with fetal circulation, and fetuses after injection of labelled iron sorbitol in pregnant rats.

Values given are percentages of doses administered to the mother.

Placenta I: without fetal circulation.
Placenta II: with normal fetal circulation

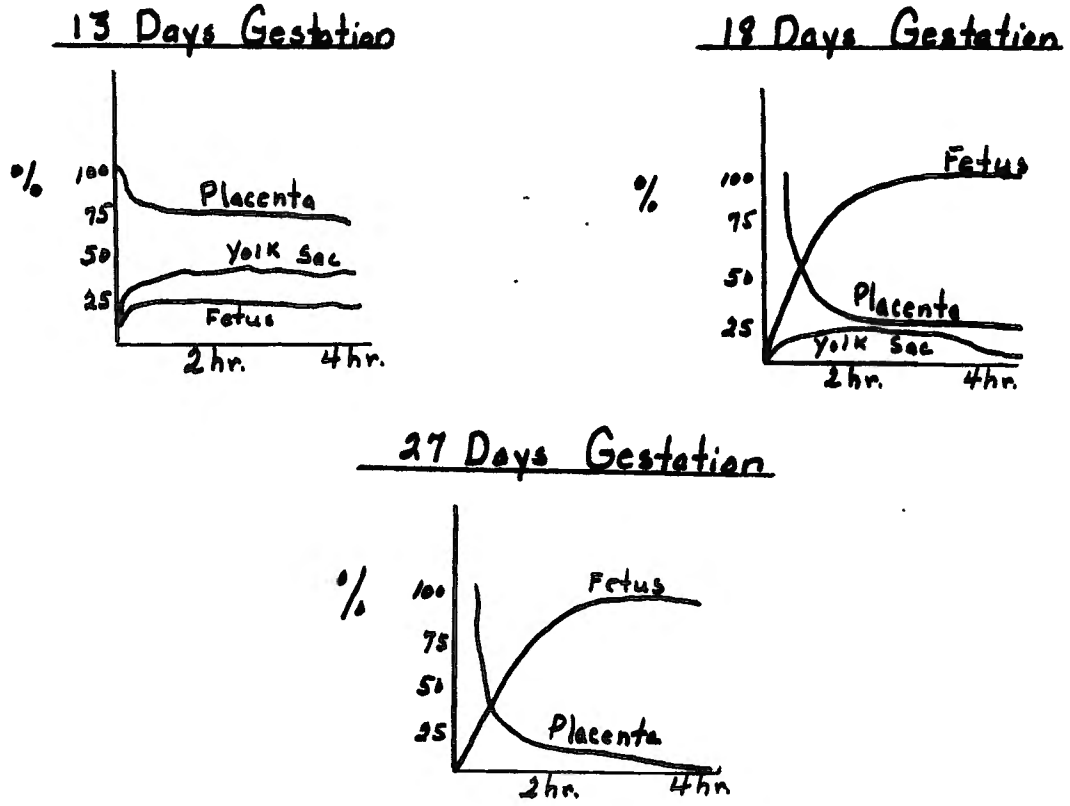
Days of Pregnancy	Organ	Fe-59%	
18	placenta I	0.38	
	placenta II	0.18	
	fetus	0.56	0.74
19	placenta I	0.68	
	placenta II	0.12	0.86
	fetus	0.74	
20	placenta I	0.71	
	placenta II	0.15	1.07
	fetus	0.92	

(Figure 8)[11]

Activation Analysis as Used to Study Iron Metabolism

As mentioned earlier in this discussion of iron metabolism, one of the stumbling blocks to further investigation has been the lack of an adequate means to follow ferrokinetics during human pregnancy. We feel this difficulty can be overcome by the use of activation analysis. Basically, activation analysis consists of bombarding an element with neutrons and causing a nuclear fusion and producing a new isotope or element. The isotope is radioactive, it will decay and emit gamma rays. These rays can be detected by such apparatus as Geiger - Meuller Tubes. By counting the characteric emissions given off by the radioactive elements, they can be identified. Activation analysis in tracers is carried out with a stable yet rare isotope of the

Percent of Radioiron in Rabbit Fetuses



(Figure 9)[13]

Iron Distribution in the Fetal Complex

Trimester	Fetal Fluid and Placenta	Fetus
1	85.9%	14.1%
2	57.7%	42.3%
3	31.7%	68.3%

(Figure 10)[13]

element whose metabolism is to be studied. After the element has been passed through the desired cycle, a sample of the product in question is irradiated by neutron bombardment. Then, since the rare, stable,

isotope of the element is of a different nuclear configuration than the majority of the element in its natural state, it will be made radioactive at a different rate than the naturally abundant form of the element. The radioactivity can then be counted in a multi-channel analyzer, and by comparing the peaks found in the sample to those of a standard, a quantitative estimation of the element in the sample can be determined. Thus the metabolism of a trace element can be studied without subjecting patients to radioactivity.

Activation analysis was originally used in 1938 for determination of trace elements in ore samples. However, the first use in biological systems was not until 1956 when it was used to determine the quantity of electrolytes in muscle and serum.

Dr. J. T. Lowman from the University of Minnesota has written two papers in which he has used activation analysis to determine plasma clearance rates of iron in human subjects.[14, 15] In these papers Dr. Lowman sets forth criteria which are necessary for the use of activation analysis in kinetic studies such as those we propose. These criteria are as follows:

1. The isotope of the element to be studied should not occur naturally in the sample, or if it is present the element should be present in minute quantities. In this way the exogenously administered isotope can be distinguished from that naturally present.

2. The isotope must have the physical ability to be made radioactive by neutron bombardment at a faster rate or to a different radioactive isotope than the naturally occuring element.

3. The added tracer isotope must be at a sufficient concentration to be detected by the available equipment.

4. The isotope must be activated in direct proportion to its concentration in a sample; and any subsequent sample exposed to the same beam of reactor neutrons for an identical period of time must become activated to the same extent.

5. The irradiation time needed for counting a significant number of gamma emissions must be short enough such that results can be obtained in a reasonable length of time. The counts per unit time can be determined by the following formula:

$$A_t = N\ f\ \sigma\ \ (1-e^{\ .693+/T_{\frac{1}{2}}})$$

where: A_t = disintegrations per second where time (t) is the given time of activation.

N = the total number of nuclei of the element present in the sample.

f = the number of thermal neutrons present in the reactor/cm^2/ sec.

= the probability for activation of a given isotope.

$T_{\frac{1}{2}}$ = the half life of the radioisotope produced.

6. Activation analysis requires that an easily detectable disintegration be produced by the activated isotope. In order to be quantitated accurately, the isotope should be chemically separated from other compounds in the sample or the gamma emissions should be separated by the use of specific pulse height analyzers.

When used in iron metabolism, the isotope of iron selected is Fe-58. This isotope was selected because it has only a natural abundance of 0.33%, and on neutron bombardment it is readily converted to Fe-59. Second, Fe-59 has two characteristic gamma emissions at 1.10

and 1.30 mev. which are in a moderately high range such that they can be readily separated from most of the other elements giving lower levels of gamma irradiation. Third, Fe-59 has a half life of 45 days, and two of the interferring elements with similar emissions, Na-24 and K-42, have half lives of 15 and 12.5 hours respectively. Therefore, by letting the sample decay several days, the activity of these elements becomes insignificant. Finally, the counts per second given off by Fe-59 are enough to be detected with readily available equipment, without counting the emissions for an excessive length of time.

Dr. Lowman[15] has shown that Fe-58 can readily be used to determine plasma clearance rates of iron in humans. In our investigations we wanted to determine if it was possible to detect quantities of Fe-58 which we had calculated to be in the cord blood and serum of newborns. If these experiments are satisfactory, we feel that activation analysis with Fe-58 would provide a useful tool for further ferrokinetic studies in pregnancy.

Materials and Methods

The experimental solution was prepared in the following manner: Fe-58 in the form of Fe_2O_3 was purchased from the Oak Ridge National Laboratory. The iron oxide was 85% enriched as Fe-58. The iron oxide was dissolved in concentrated hydrochloric acid with ascorbic acid added to reduce the iron from the ferric form to the ferrous form as $FeCL_2$. The ferrous chloride was then diluted with distilled water to give a concentration of 4.58 ugm. Fe-58/ml. $\pm$ 2%. This concentration was chosen based on the theoretically calculated

concentration of iron that could be found in 10 ml. of cord serum if a 250 ugm. tracer dose of Fe-58 were administered to the mother intravenously at lease 4 hours prior to delivery.

Three 1 ml. samples were then irradiated for 1, 4, and 8 hours respectively to determine what would be the optimum time for irradiation in order to get a significant number of gamma emissions from the iron sample.

Following the calculation of the standards, which consisted of 1 ml. of the standard solution in 5 ml. distilled water, the Fe-58 solution was added to whole blood and serum. Before the Fe-58 solution was added to the blood and serum the protein had to be hydrolyzed from the sample to prevent excessive radiation counting of radioactive protein. This hydrolyzation was carried out by adding 10 ml. of the serum or blood to 10 ml. of concentrated nitric acid. These solutions were then heated for 10 to 12 hours until the liquid was evaporated. The remaining mixture was then redisolved in 4.0 ml. nitric acid and 1 ml. of the standardized solution of Fe-58 was added to a sample of serum and whole blood. A second sample of whole blood was then put through the same process of hydrolysis and was redisolved in nitric acid but was not spiked with the Fe-58. Thus samples of enriched whole blood, enriched serum, nonenriched whole blood, a standard sample, and a blank sample containing only distilled water were used. Each of these samples was irradiated for eight hours in the Triga nuclear generator of Omaha Veterans Administration Hospital for 8 hours, and the samples were counted in a 400 channel 4 mev. pulse height analyzer for ten minutes at 12, 13, 16, and 23 days decay following irradiation. We chose to use the

Irradiation time	Counts/10 min. at 1.10 mev.	1.28 mev.	Summation Counts (105-115)	(123-133)
1 hr.	21	11	156	96
4 hr.	63	35	572	343
8 hr.	118	54	1130	672

(Figure 11)

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peaks of gamma emission given off by Fe-59 at 1.10 and 1.28 mev. since the peaks at lower voltages were obscured by irradiations from contaminating elements such as Na, K, and Cl. The higher Fe-59 peaks were also not included because the number of counts given off at these levels by 8 hour irradiation and detected by 10 minutes of counting were not statistically significant. Besides counting emissions at the peak, a summation can be made in the 5 channels on either side of the peak since the majority of these emissions will also be from the Fe-59.

Results

The 3 samples irradiated for 1, 4, and 8 hours were used to determine the optimum time for irradiation and counting. The following results were obtained counting after a 3 day postirradiation decay period (Figure 11). It was decided from this data that 8 hours irradiation would be the optimum time for irradiation since a distinct peak can be seen in the desired channels and irradiation longer than 8 hours would only necessitate a longer decay time to get rid of interfering emissions.

Next, samples of serum and blood were irradiated for 8 hours and counted with the following results (figures 12,13,14,15,16). Figures 17,18, and 19 are graphs of the results presented in the previous five tables.

Sample	Summation Counts/10 min. (105-115)	(123-133)
Blank	42	9
Standard	820	453
Serum spiked	1160	760
Whole blood spiked	3225	1518
Whole blood not spiked	2603	1255
	counted 10/30/67	irradiated 10/18/67

(Figure 12)

Blank	25	8
Standard	788	457
serum spiked	837	467
Whole blood spiked	2935	1350
Whole blood not spiked	2264	1014

counted 10/31/67

(Figure 13)

Blank	27	11
Standard	731	413
Serum	616	336
Whole blood spiked	2639	1161
Whole blood not spiked	1906	834

counted 11/3/67

(Figure 14)

Blank	27	11
Standard	865	322
Serum spiked	727	239
Whole blood spiked	2990	824
Whole blood not spiked	2180	586

counted 11/10/67

(Figure 15)

Sample	Whole blood minus spiked	Whole blood not spiked	Serum		Standard	
	1.10 mev.	1.28 mev.				
10/30/67	622	263	1.10	1.28	1.10	1.28
10/31/67	671	336	1160	760	820	453
11/3/67	733	327	837	467	788	457
11/10/67	810	238	616	336	731	413
			727	239	865	322

(Figure 16)

Discussion

Discussion on iron metabolism in pregnancy has been presented in the first portion of this paper. This was presented not only as a short literature review, but also to point out the need for further study in this area, particularly concerning placental iron transfer. It has been shown that activation analysis, using Fe-58, can provide a new and necessary tool for study of ferrokinetics in pregnancy. Our results show first of all that Fe-58 can be detected in trace amounts such as would be expected in the fetal circulation. We have found that for quantitative analysis using Fe-58, an 8 hour irradiation period followed by a 16 day decay time is satisfactory. Also, for our quantitative determinations the peak at 1.10 mev with a summation peak count between 105 and 115 mev. gives the best results. Using these criteria, the ratio of spiked minus nonspiked blood to the standard counted at 16 days was 733/731 and at 23 days was 810/865. Both of these values are within a 93% correlation with each other. This would indicate that this method could be used satisfactorily to quantitate the iron in a sample. Serum values did not correlate as closely with the standard. However, this is most probably due to a loss of solution during preparation rather than to a fault in the method itself. It is also important to note that with the proper decay time a serum control does not have to be run simultaneously with the standard and serum sample. This is because the elements present in serum have a short half life as compared to those in blood and do not cause interfering peaks in the ranges of Fe-59 emissions.

In spite of these results the method of activation analyses is not without certain difficulties. The primary problem is substances besides those being determined which become radioactive and give emissions in channels close to those of Fe-59. In previous work done with activation analysis using Fe-58 and whole blood it was felt that metastable Co-60 had interfered with the iron peaks since cobalt gives peaks of gamma emission at 1.17 and 1.33 mev. 33, 34. In the present study cobalt was shown not to be an interfering substance. This was done by recounting the samples after waiting for at least one half life of Fe-59, 45 days. By doing this it was felt that if there was not a significant decrease in the number of peak counts that cobalt was interfering because its half life is greater than 5 years and 45 days decay should have little effect on its emissions. Thus it is seen that cobalt is not an interfering substance at the 16 day count for the following reasons: First, besides the 2 peaks already mentioned for cobalt, it has a summation peak at 2.5 mev. If Co-60 is interfering this peak would be present in our counts; however, no such peak could be seen in either blood or serum samples. See figure 14. Second, a close correlation has been obtained between the standard and spiked samples and cobalt is not present in the standard sample.

Summary and Conclusions

The review of the literature which was presented shows that the current knowledge of iron metabolism in pregnancy is inadequate and controversial. Particularly inadequate are detailed studies on iron metabolism during human pregnancies. This is because the most ef-

fective agent for study of iron metabolism if Fe-59. This element, if used to study the human being during pregnancy, might subject the fetus to undesirable irradiation. Yet, as can be seen, further studies are needed regarding iron absorption in pregnancy, iron utilization in the mother, placental iron transfer, and iron storage and utilization in the fetus.

A method has been described and the techniques developed and described in vitro for the measurement of trace quantities of iron in serum and whole blood by activation by activation analysis. This method shows great promise as a second tool for two reasons: First, iron metabolism can be studied in the human without subjecting patients to irradiation. Second, since Fe-58 is a stable isotope, injected iron can be studied in a human at any time in the future--weeks, months or even years because the isotope does not decay.

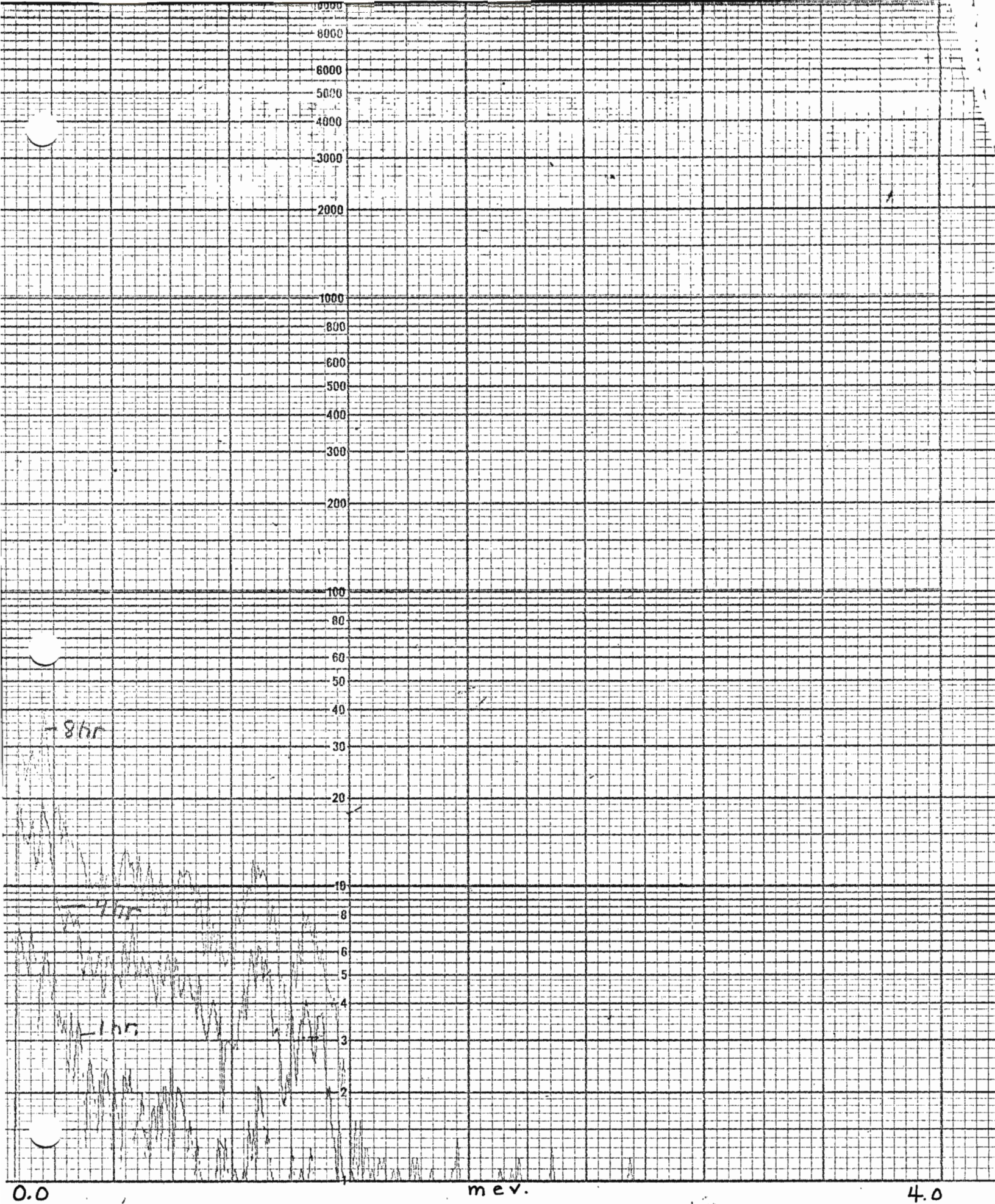
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Figure 17

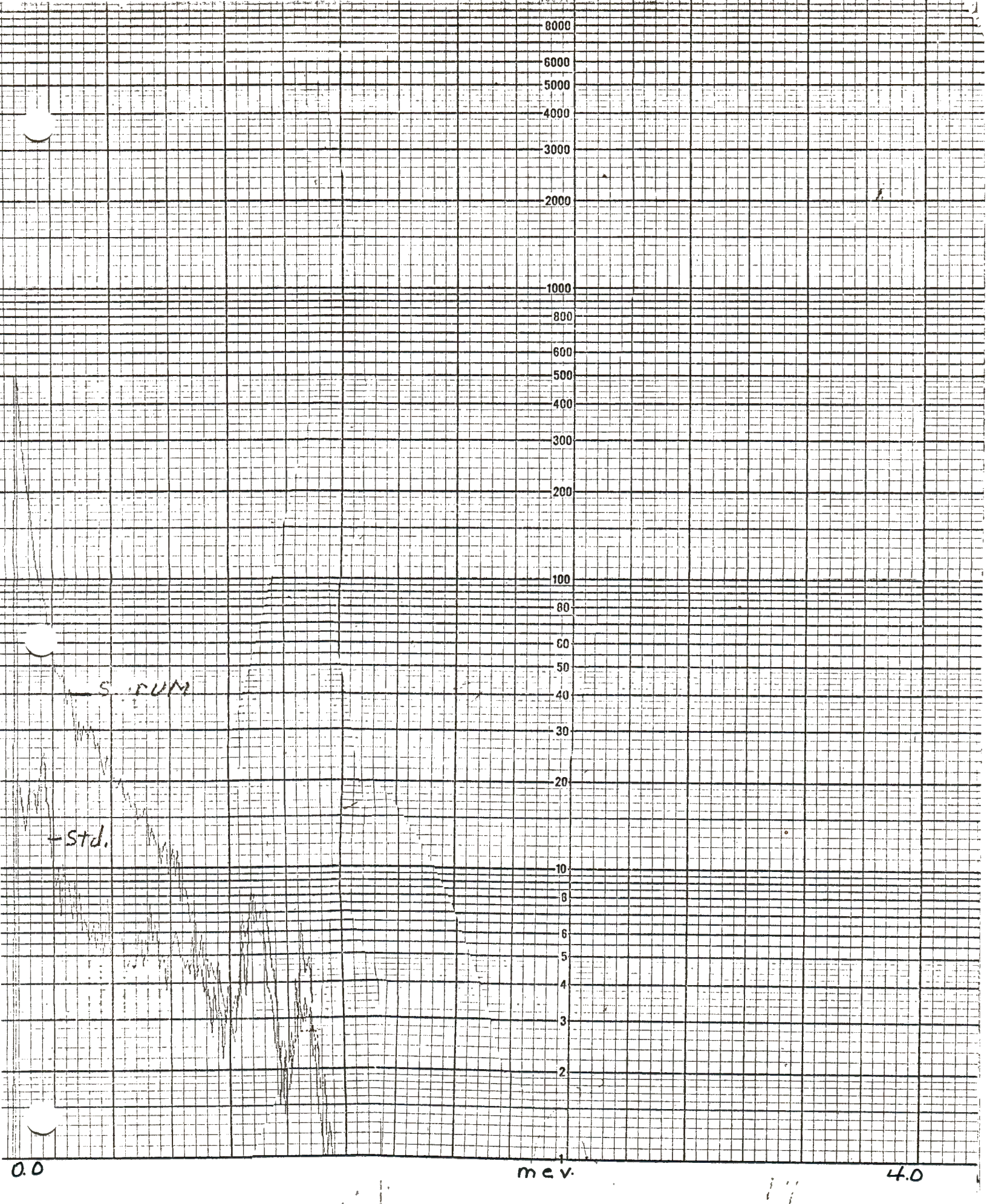
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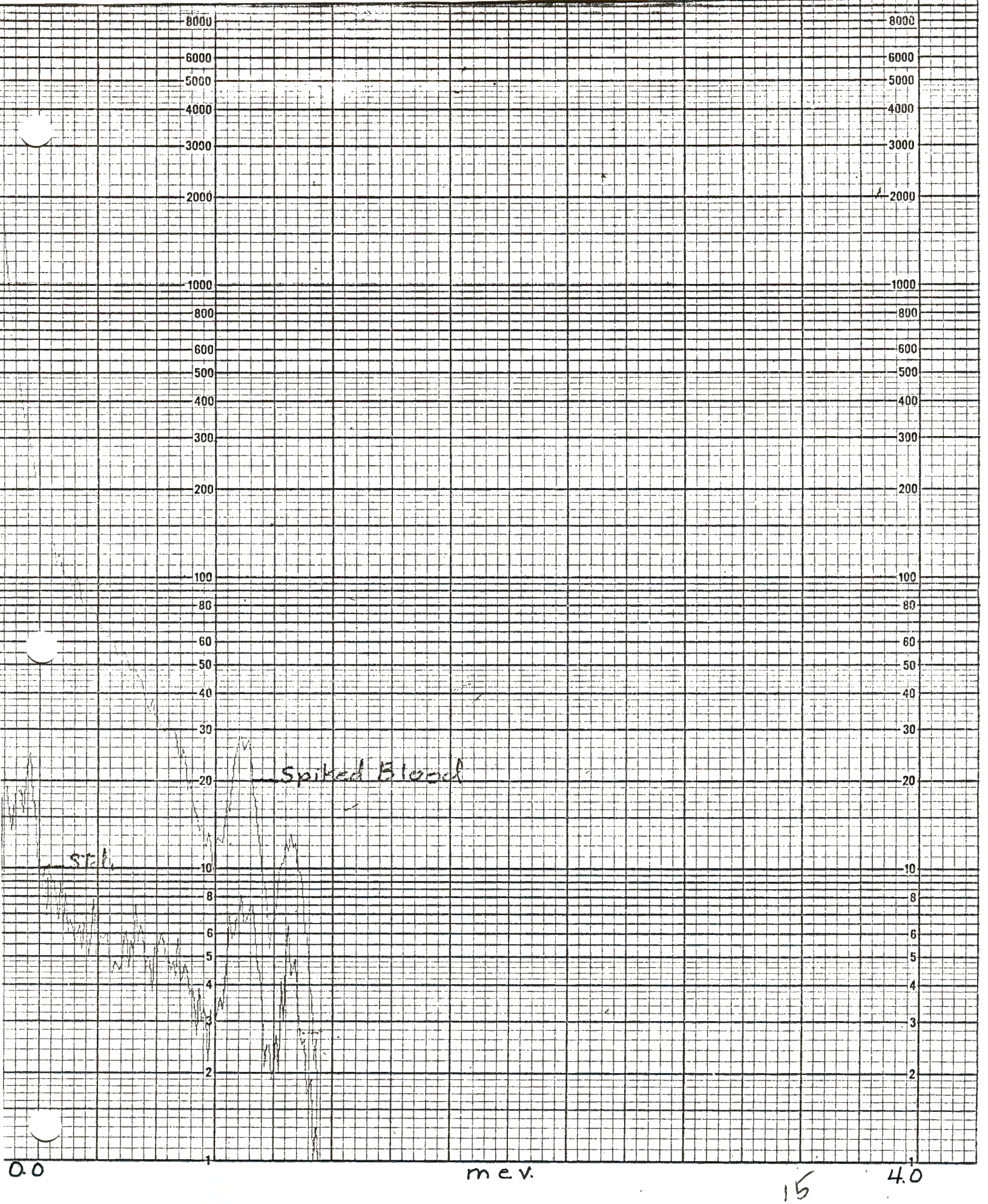
Figure 18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2
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Spiked Blood
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mev.
4.0

Figure 19

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