AN ABSTRACT OF THE THESIS OF

James Edmund OLDFIELD (Name) for the PhD (Degree) in Animal Nutrition (Major)

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Title Iron Deficiency Anemia as a Contributory Cause of Early Mortality in Swine.

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Mortality in young pigs during the first few weeks of post-natal life has proven to be the most troublesome single problem facing swine producers in this country. Numerous investigations have been undertaken in attempts to correlate these losses with some specific disorder. A review of the agricultural literature together with observations made on both sickly and deceased young pigs has pointed to iron deficiency anemia as an important contributor to such early mortality. It would appear that young pigs, because of their extremely rapid early post-natal growth together with the low iron content of their mothers' milk, are particularly susceptible to a physiologic anemia of the iron deficiency type.

A survey of the literature reveals that iron absorption is the dominant process in the metabolism of that metal, and that the efficiency of absorption may vary widely under different conditions. Various workers have shown conclusively that absorption of iron is most efficient when a definite need for the metal exists in the body, such as for the synthesis of hemoglobin. This condition has suggested the possibility that iron might be efficiently taken up by a sow during pregnancy, when the requirements for the growing feti are considerable. The efficiency of such iron uptake might conceivably be measured by the blood picture and the extent of iron storage in the young.

Preliminary experimentation was undertaken to demonstrate the presence of anemia (as reflected in hemoglobin levels) in young pigs raised under dry lot conditions, and to evaluate the non-hemin iron stores of the livers of the young animals. A Spencer hemoglobinometer was found to be a convenient apparatus for the measurement of blood hemoglobin, and correlation of determinations made with it against iron content of the samples used indicated a reasonable degree of accuracy. The length of time involved in carrying out the appropriate experimental observations with pigs limits the number of experiments that can be performed, and the use of rats as "pilot" animals might seem advantageous. It was found impossible however to demonstrate

a condition of anemia in young rats similar to that in young pigs, even when the rats were raised under rigidly controlled conditions.

Consideration of the therapy employed routinely in human pregnancy anemia has suggested that high dosage levels of iron (above 100 mg. daily) might be effective in raising the iron stores of young pigs pre-partum, where smaller doses have failed. Administration of high levels of ferrous sulphate and of molybdenized ferrous sulphate to sows was followed by maintenance of hemoglobin levels above the anemic state in the young, but no reflection was noticed in the form of sizable non-hemin iron stores in the liver. The magnitude of liver iron stores encountered practically precluded the possibility of such stores being valuable in the prevention of anemia in young pigs. The use of molybdenized ferrous sulphate raised an interesting point, as no apparent toxicity developed, nor interference with the copper-iron relationship, even in the face of extremely high levels of administration. The nature of any molybdenum - iron relationship remains obscure, and is indicated as a topic for future study.

IRON DEFICIENCY ANEMIA AS A CONTRIBUTORY CAUSE OF EARLY MORTALITY IN SWINE

by

JAMES EDMUND OLDFIELD

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APPROVED:

Redacted for privacy

Chemist (Animal Nutrition)

In Charge of Major

Redacted for privacy

Head of Department of Animal Husbandry

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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IRON DEFICIENCY ANEMIA AS A CONTRIBUTORY CAUSE OF EARLY MORTALITY IN SWINE

INTRODUCTION

Early death losses in young swine, often occurring within a few days of birth, are of such a magnitude as to pose the most important single problem facing swine growers in this country at the present time. As an example, studies conducted by the writer on farrowing records in the Oregon State College herd show that there has been an average mortality between birth and weaning (about eight weeks of age) of 32 per cent of the animals born in recent years. Reference to the agricultural literature indicates that early losses as high and often higher are common across the country. When one realizes that existing records are compiled almost entirely at Experiment Stations, Colleges, and other institutional establishments where the standard of management and nutritional practices is above average, it becomes evident that country-wide losses of this type might well include four-tenths of all pigs farrowed. Such wastage is indeed staggering. Economically it means that the country is deprived of a large proportion of its potential meat supply - a factor of no mean importance in view of the unsettled nature of international affairs today.

Obviously, losses of the magnitude described could not be ignored by either the producers themselves or by those interested in research in agriculture, and indeed they have come in for very serious consideration and extensive study. It may be well, by way of introduction to review some of the earlier work in the field of baby pig

mortality, and to note some of the reasons which have been advanced for an association of such losses with anemia.

As early as 1890, Braasch, in Schleswig-Holstein (40) noted a very heavy mortality rate in young pigs raised under conditions of intensive production, in connection with dairy enterprises. Initial appearance of heavy losses seemed to coincide with what was then a new practice, that of raising young pigs in central colony houses, without free access to the soil. Braasch apparently recognized the incidence of anemia among the animals he observed, but he attributed the deaths to a failure of "modern" management practices to meet the physiologic requirements of the pig. Probably the first allusion to nutritional anemia as a serious disorder in young swine was made by McGowan and Crichton (133,134) in 1923. These Scottish workers demonstrated the presence of severe nutritional anemia in young pigs born in certain breeding establishments where sows were farrowed on concrete floors and fed only a dry ration. It was noticed that after apparently normal farrowing the young pigs would grow in a satisfactory manner until 3-4 weeks of age, after which they took on a "stocky" appearance due to edema under the skin. Death was common where treatment was not resorted to, and often whole litters perished. Similar conditions occurring in this country were recorded in the literature by Doyle, Mathews, and Whiting, (40) who were able to show a high degree of correlation between anemia and death losses in young pigs. Craig, also, in a review including observations on 277 young pigs (31) noted a mortality of 60.5 per cent among those described as anemic, compared to only 3.8 per cent among non-anemic animals.

These observations from the scientific literature indicate an early cognizance on the part of some investigators of the possible implication of anemia in early mortality in swine. On the more practical level, there has always been an opinion widely held among stockmen that most losses in young pigs are the result of accidental crushing by the sow. Certainly a very large proportion of the deaths occur during the first week of life, when crushing might well take place. Two interesting theories, based partly on observation and partly on conjecture, may be advanced in an effort to explain how such crushing might occur. In the first place, in modern farrowing pens where the young pigs have the advantage of a guard rail for their protection, one might suppose that they would normally have a good chance to escape as the sow lies down. If, however, the vitality of the young animals is lowered through some physiological abnormality, the chances for escape might be greatly reduced. It seems quite possible that anemia, through its influence on the processes of respiration, might well be the physiological abnormality involved. In the second instance, as the actual death of young pigs is seldom observed, it is possible that crushing may have occurred post-mortem, and that some other factor was, in effect, the cause of death. One may argue that, as the hemoglobin content of the blood of pigs is essentially normal at birth, (41) it is doubtful whether an anemia could reach a fatal degree of severity in such a short time. This may be true, but the possibility must not be ignored that anemia may lower the resistance of the young animal to other disorders, perhaps of an infectious nature, which could in turn be the primary cause of death. Suggestions posing a

contributory effect between swine anemia and pneumonia may be found in the veterinary literature (78).

The foregoing introduction has presented some aspects of the problem of baby pig losses insofar as these losses may be contributed to by nutritional anemia. It should not be assumed that this complex and extensive problem may be laid to any one discrete cause, in fact its eventual solution will probably be the result of investigations in various fields, including those of disease, management practices, and nutrition. Some instances of other probable causes of baby pig loss may be cited in order to give a broader picture of the situation. Young and Underdahl (210) for instance, at the Hormel Institute, demonstrated some lowering of mortality among young pigs whose dams had been vaccinated against influenza during pregnancy. On the other hand Adsersen (3) has observed over a number of years that mortality in suckling pigs may only exceptionally, if at all, be ascribed to infection. Routine vaccination with swine Pasteurella preparations did not prove effective in lowering these losses. Graham, Sampson, and Hester (72) reported conditions of hypoglycemia in newly born pigs which was so striking that they titled their paper "Acute Hypoglycemia - (So-Called Baby Pig Disease)." Hypoglycemia, while it may be severe, however, often cannot be demonstrated at all, and it seems proper to place such appearances of low blood sugar among the symptoms of a contributory, rather than a major cause of baby pig loss.

In contrast to the preceding two instances, conditions of nutritional anemia have been extremely widespread where young pigs

have been farrowed indoors and have spent the early weeks of their lives without access to the soil. References to such conditions are plentiful in both the scientific and popular literature, and mention will frequently be made to them in the discussion to follow. The purpose of this thesis as embodied in the title is to investigate the relationship between nutritional anemia and early mortality in swine. However, such a purpose may be strengthened by the additional investigation of the many cases where nutritional anemia, although not fatal, contributes to an impaired physical condition and lowered growth rate in the animals concerned. Some will argue that the question of baby pig anemia has already been adequately investigated, and that suitable curative methods are presently at hand. It is true that successful treatments have been developed, however further work in this field seems justified by two facts: the continued prevalence of nutritional anemia in young pigs, and the unabating severity of death losses among these animals. It would seem that the methods of treatment developed, although theoretically efficient, have not always been acceptable in practice. Consequently the objective of this thesis becomes an investigation of the ramifications of nutritional anemia in swine, and an attempt to provide a preventative method, suitable for practical application, by which the disorder may be combatted.

NUTRITIONAL ANEMIA IN SWINE

General Description and Symptomatology.

"Anemia" is a term having a very extensive meaning, involving any condition which results in an imbalance between the formation and destruction of blood. It is commonly applied to any deficiency in quality or quantity of blood, such as may be manifested by reduction in the number of red cells, amount of hemoglobin, or both (1). Such a description immediately suggests a divisional classification of anemias into those concerned with decreased blood formation, and those which come into being through increased blood loss. Excellent comprehensive classifications of the various anemias may be found in many texts (79, pp.214-215; 15, pp.61-69; 61, p.79) and it is only necessary here to designate in some manner the type of anemia concerned in this study. Most commonly, the anemia of young pigs is characterized by a lowered hemoglobin content in the blood, in conjunction with a lowered proportion and size of erythrocytes. This combination of factors produces a blood of lighter than normal colour, and the anemia is termed hypochromic and microcytic. Further, as no marked loss of blood by the young pigs is discernible, the anemia is properly classified as one of deficient blood formation, or failure of hematopoiesis to keep up with the increasing requirements of the rapidly growing animal. Such an anemia has come to be recognized as characteristic of a nutritional deficiency of one or more of the blood-forming elements, most commonly iron.

Numerous descriptions of the anemia occurring in young pigs exist in the literature, and it is possible from these to outline a syndrome of symptoms. Under gross outward symptoms of the disorder, McGowan and Crichton (133) and later Kernkamp (115) have noted a plump and stocky appearance of the body, especially in the region of the head and shoulders. (This observation may help to explain the common belief that anemia often takes the largest and "best" pigs of a litter.) The pigs often have a dull, listless attitude, and frequently shake and shiver as if chilled. Visible mucous membranes around the nostrils, eyes, and mouth are extremely pale. Respiratory movements, particularly during later stages of anemia, are often quite irregular, involving jerky, thumpy movements of the diaphragm, and it is this condition which has given rise to the popular nomenclature for piglet anemia of "Thumps."

The most striking post-mortem finding in anemic pigs is the presence of large quantities of straw-coloured edematous fluids, which often nearly fill the pleural cavities. The heart is usually considerably enlarged, and is soft, pale and flabby to the touch. Characteristic lesions occur in the liver, which is enlarged, very friable, and mottled due to a process of fatty infiltration(40,136). As far as the blood is concerned, it appears that the young pigs are born with a normal hemoglobin level and erythrocyte count, but both these components become significantly lowered during the early weeks of life if not type of treatment is employed. The hemoglobin content of the blood of young pigs raised outdoors and in confinement is given, from the literature, in Figure 1, illustrating the sharp progressive difference which first

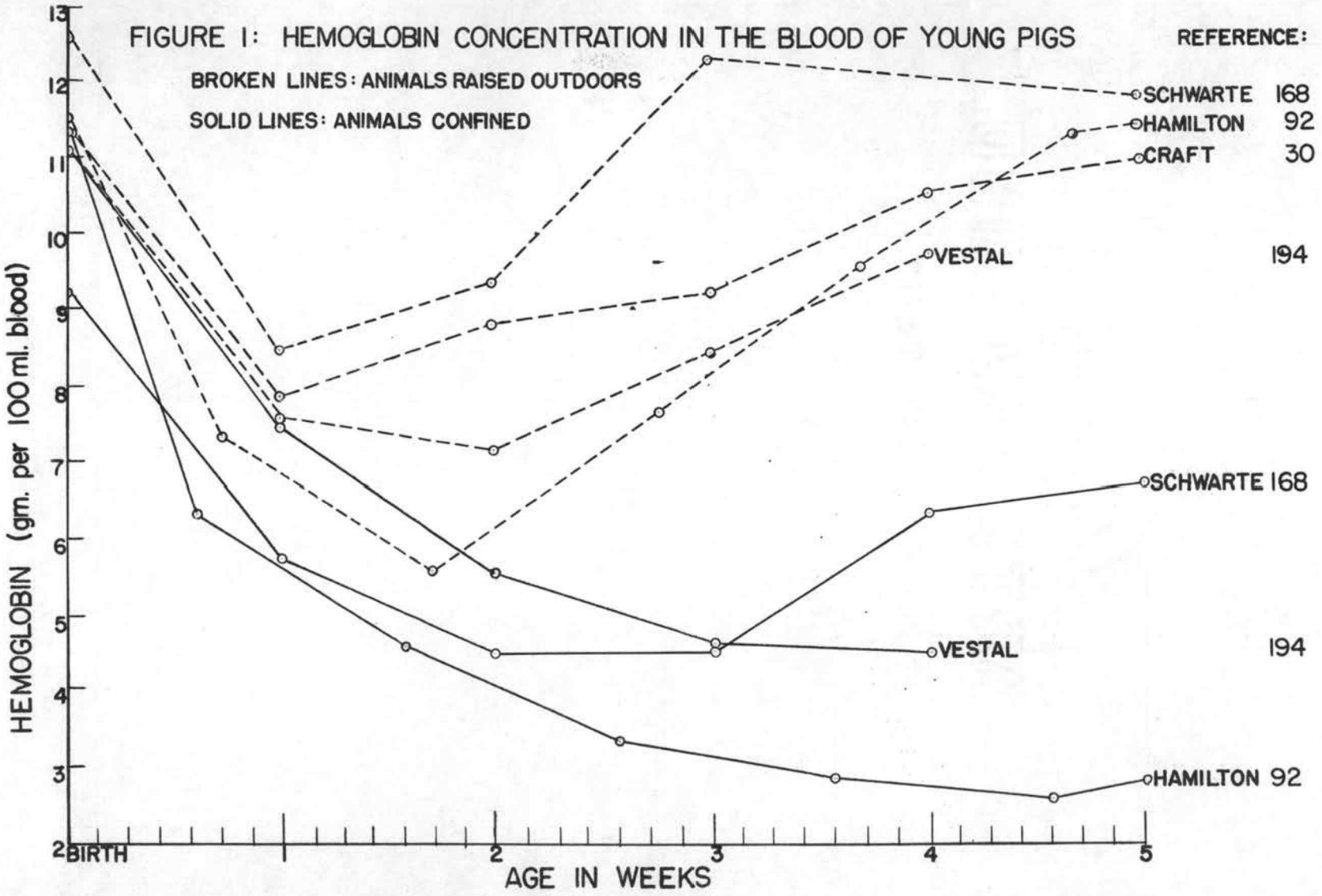


FIGURE 1: HEMOGLOBIN CONCENTRATION IN THE BLOOD OF YOUNG PIGS
BROKEN LINES: ANIMALS RAISED OUTDOORS
SOLID LINES: ANIMALS CONFINED
REFERENCE:
SCHWARTE 168
HAMILTON 92
CRAFT 30
VESTAL 194
SCHWARTE 168
VESTAL 194
HAMILTON 92
HEMOGLOBIN (gm. per 100 ml. blood)
13
12
11
10
9
8
7
6
5
4
3
2
BIRTH
1
2
3
4
5
AGE IN WEEKS

attracted attention to this field of study. It is interesting to note that some lowering of the hemoglobin level occurs even in apparently "normal" pigs raised under farm conditions, (outdoors) and that considerable variation in hemoglobin levels among members of the same litter may be apparent (168). Furthermore, the progressive decline in hemoglobin has been observed to proceed at different rates by various investigators - some evidence of this is given in Figure 1. It is fairly generally accepted that a hemoglobin level of less than 6 grams per 100 ml. of blood constitutes clinical anemia in the pig.

The erythrocyte count in the blood of young pigs follows a course at least partly parallel to the hemoglobin level. Again, there is a decline under all conditions during the first few weeks of life, the extent of which is increased by confinement of the animals. Palmer (154) found an average red cell count of 3,900,000 per cu.mm. in 25 pigs ranging in age from 2-42 days, as compared with 6,200,000 in 25 pigs weighing over 100 pounds, (likely over 120 days of age). So-called "normal standards" for the erythrocyte content of pig blood have been set a 2-9 million per cu.mm. by Fraser (64) and Scarborough (163), but such figures are of questionable value because of the diverse ages of the pigs involved. Burnett (23, p.58) cites data illustrating a lower normal erythrocyte count in young than in adult pigs. Hemoglobin and erythrocyte levels have been compared by Klein and Kuhn (119) who observed that the minimum level for hemoglobin was reached at 21 days of age, while that for erythrocytes occurred at 10 days. Such a description would indicate a condition of microcytosis in the later stages of anemia. It seems to be the opinion of most workers in the field

that among the blood constituents examined, hemoglobin was most valuable in diagnosis of anemia, and its determinations were most accurate.

Etiology of Anemia In Swine.

It may be well in approaching a discussion of the etiology of anemia prevalent in baby pigs to review some of the pertinent early investigations. Probably the first recorded observation in this field, as noted in the introduction to this thesis, (40) was that the anemia occurred frequently among animals confined in concrete-floored pens, and was conspicuously absent among animals running on pasture. Obviously, those animals allowed access to the outdoors were obtaining something necessary to the optimum function of their hematopoietic systems - something which the confined animals were not. The nature of the factor concerned was not immediately apparent, nor whether it was supplied through the sunlight, herbage, or soil, any of which would be available to animals outdoors but not to those confined. It is interesting to note some of the early work which was carried out, (not always with direct reference to swine) enabling later investigators to narrow down their field in the search for the active factor involved.

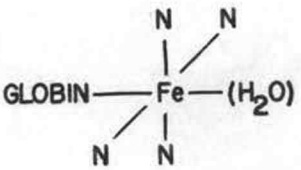
It was perhaps natural that sunlight should come in for careful consideration as a possible anti-anemia factor. Sunlight had been recognized from ancient times as an important contributor to health and well-being in humans and animals alike, a recognition that was strengthened scientifically in the early 1920's by the correlation by various workers of the effects of sunlight and vitamin D (107,179). The case

for an effect of sunlight with respect to anemia grew out of the observation of the pallid, anemic appearance of miners and other workers who were habitually denied the benefits of the sun's rays. Laurens, (124) however, in his review on the effects of radiation, suggests that this pale appearance does not necessarily reflect a change in blood composition, but rather a decrease in the total volume of blood circulated to the periphery. It is thought that light, within certain physiological limits, dilates the cutaneous blood vessels, thereby increasing the amount of blood circulating in the skin area. In contrast to the very empirical evidence for occurrence of anemia in the absence of direct sunlight, it should be mentioned that members of polar expeditions exposed to long periods of darkness or semi-darkness showed no evidence of anemia as long as their food supply remained adequate (124). Direct investigation with swine later confirmed the theory that ultra-violet light, per se, had little or no effect on the incidence of piglet anemia. Fulton at Saskatchewan (68) illustrated that litters of pigs maintained on concrete but with access to direct sunlight developed anemia to a similar degree as others denied sunlight. Mathews, Doyle, and Whiting (136) were likewise unable to demonstrate any beneficial effect on hemoglobin production in young pigs through irradiation, with a mercury vapor lamp, of either the sow, pre-partum, or the litters up to 35 days of age.

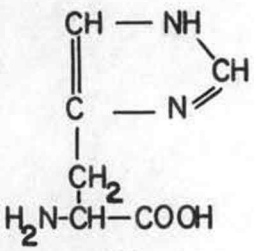
Concurrently with the investigation of the possibility of effects of radiation in anemia, numerous workers were exploring the field of nutritional relationships, and such studies of course, centered about substances necessary for the production of hemoglobin.

FIGURE 2: HEMOGLOBIN AND RELATED SUBSTANCES:

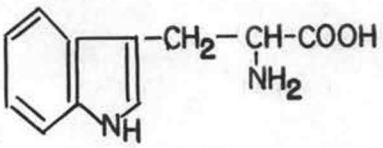
Hemoglobin is a conjugated protein consisting of Globin and a non-protein part, Heme. In the diagram to the right the structure of hemoglobin is shown, with the 4 nitrogens of the pyrrole rings representing the porphyrin ring. The nature of the Heme to Globin linkage is not definitely known, but apparently occurs through the Heme iron, and may involve imidazole rings in the Globin. If such is the case, the amino acid Histidine might be involved. Other structures that might be concerned in this linkage are the indol grouping of Tryptophan, or a Pyrrolidine group as in Proline.



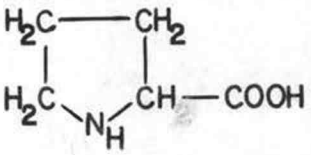
HEMOGLOBIN



HISTIDINE

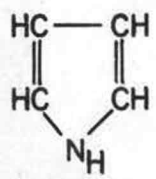


TRYPTOPHAN

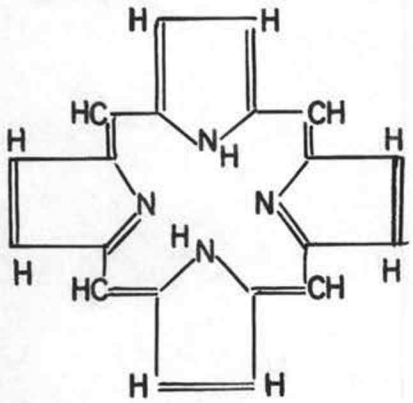


PROLINE

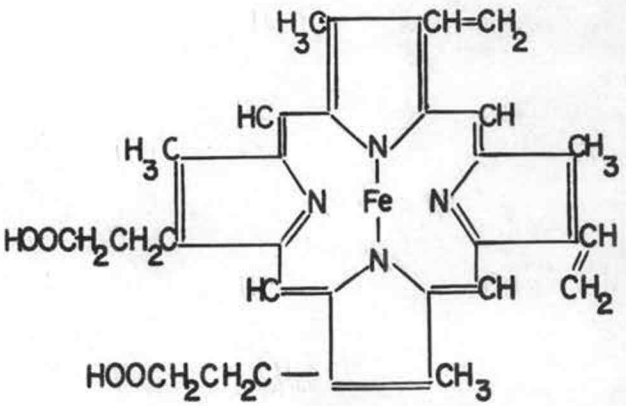
Hemoglobin as such exists only near the neutral point, and acid may cause denaturation of the Globin, freeing the iron-containing portion. Breakdown products of Heme have all been found to be derivatives of Pyrrol. Four of these Pyrrol rings may be linked to form the cyclic ring system Porphyrin. The actual prosthetic group of hemoglobin, which may be split off by the action of acids, is Proto-heme, an iron-porphyrin.



PYRROL



PORPHYRIN



PROTOHEME

REFERENCES: Anson and Mirsky (6,7) Haurowitz (99, p. 206,208)

The essential materials for hemopoiesis are iron, the pyrrole nucleus, either as protoporphyrin or in some simpler form, and the constituent amino acids of globin, as portrayed in Figure 2. Perhaps it may be permissible here to depart from the chronological order and discuss first some of the later work concerning the non-ferrous blood components. In this connection, one is faced with the possibility that a limiting factor in hemoglobin production might be a deficiency of either the pyrrole or globin portions of the molecule. In 1939, Kirkman, (118) who investigated the relationship of porphyrin deficiency in the diet to rate of erythropoiesis, came to the conclusion that it had not a consistent importance, at least under a normal, practical dietary regimen.

Because of its similarity to protoporphyrin, chlorophyll in green plants might well be an important substance for hemoglobin building, but results from dietary supplementation with chlorophyll have been inconclusive in this regard(121). Whipple and his associates(202) who have probably carried out more extensive investigations in this field than any other group, sum up their findings briefly - "So far no conditions have been discovered in which the synthesis of the porphyrin nucleus becomes the limiting factor in hemoglobin formation." These workers have shown that the prosthetic group of the hemoglobin molecule is almost completely excreted as bilirubin, even in severe anemia, which would suggest that the animal body is normally able to synthesize porphyrin in excess of its requirements(100,101,102,176). It seems probable that porphyrin might be formed from pyrroles derived from cyclic amino acids, produced via the breakdown of diet or tissue

proteins. Naturally, were the diet one in which a deficiency of one or more of the essential amino acids existed, protein supply might become of exaggerated importance in combating anemia (14,106,159,160,161).

As far as globin is concerned, its importance is obvious, since it makes up about 90 per cent of the dry matter of the red blood cell. On the other hand, even though it has a particularly high content of leucine and isoleucine, globin may apparently be adequately supplied by any diet containing sufficient good quality protein (35). It seems evident therefore that although theoretically deficiencies of porphyrins or of globin components might limit hemoglobin synthesis, such deficiencies are not likely to occur under most practical conditions.

The virtual elimination of these non-ferrous blood components as substances of major importance in the prevention of anemia lays the way open for a discussion of the implications of iron. Of course, the physiological necessity for iron was realized much earlier than the investigations just related above, in fact it was popularly suspected before the presence of iron in the blood was known. Iron has long been a symbol of strength, and certain early peoples believed they could derive strength and "red-bloodedness" by drinking water in which the iron weapons of their heroes had been steeped. While we realize today that beneficial effects from such treatment must have been almost wholly psychological, it is nevertheless interesting to note the early connection in the lay mind between iron and the red colour of blood.

Some of the earlier quantitative data with respect to the nutritional importance of iron were presented by Bunge (22, pp.370-385) and his students over the period 1880-1905. In brief, the teachings of

this school were that young animals were born with a store of iron in their livers to tide them over the suckling period, when their dietary intake of iron, from milk, would be very small. Comparison of the inorganic composition of the bodies of the newborn with that of their mothers' milk showed a marked similarity, except where iron was concerned. They found that although the total iron content of the bodies of young animals increased slightly during suckling, the percentage of iron in the body sharply decreased. In other words, on a milk diet, the total iron in the animal body was not increased at a rate which would conform with the rate of tissue growth. It thus followed that unless the iron storage of the animal at birth was adequate a state of "physiological anemia" would be inevitable towards the end of the nursing period. Bunge also recognized the existence of species differences in iron reserves, and further mention of his work will be made later.

From these early studies one may turn for further elucidation of the iron : anemia relationship to the comprehensive work of Hart and his colleagues at Wisconsin in the early 1920's (44,45,95,96,97,195,196). This group, using rabbits and rats as their experimental animals, were able to demonstrate a nutritional anemia in the young if they were maintained beyond weaning solely on a (cows') milk diet. Working on the theory that iron was the deficient factor underlying the occurrence of anemia, they supplemented the animals' diets with ferric oxide, yet the anemia persisted. It was then thought that perhaps the reason for the inefficiency of inorganic iron was that some other blood-forming constituent was deficient, and consequently other food materials such

as corn meal and cabbage leaves were added to the ration, after ensuring that such additions were devoid of iron. Following this procedure, the cure of anemia in the young animals was rapid and complete. Further experimentation showed that the ashes of the feedstuffs mentioned were equally as efficacious as the fresh materials in the supplementation of ferric oxide for the cure of anemia, and this of course indicated that some inorganic material other than iron was involved. A characteristic blue colour appearing in the effective ashed materials led Hart, et al (97) to suggest that copper might be involved, and numerous investigations have since confirmed the accuracy of this assumption (32,46,197,198,199). It became apparent that, while it was not an integral part of the hemoglobin molecule, copper in minute amounts was a necessary catalyst for hemoglobin synthesis. Systematic use of other minor mineral elements in conjunction with iron as a curative treatment for anemia subsequently showed that copper was specific in this regard (12,13,123,187,190).

These experiments did much to clarify the picture of nutritional anemia, however the studies mentioned involved an experimental anemia produced by design in experimental animals. It is not always possible to transpose results obtained with one species of animals to another, therefore it remained to determine whether the anemia in young pigs was indeed of the type demonstrated. McGowan and Crichton (133,134) in their early studies believed piglet anemia to be primarily one of iron deficiency, and they attempted treatment through the supplementation of the ration of the sow with ferric oxide. Such treatment was, in a measure at least, helpful but the investigators questioned

whether they obtained it incidentally from scraps of feed or feces in the pen. In 1930 Hart and his colleagues (98) extended their investigations on nutritional anemia, some of which have been already cited, to swine. These workers took precautions which eliminated the possibility of the young pigs having access to the sows' ration, but these seemed to have no influence on the occurrence or severity of anemia. The chance that the young animals might obtain iron from the feces of their mother could not be ignored, and Hart and his associates investigated this by separating pigs from their mother at 3 weeks of age, rearing them thereafter on a cows' milk diet. Here again, no influence on the anemia was recorded. Cures were effected in all cases by the use of ferric chloride, administered orally to the young animals in amounts to supply 25 mg. of iron per pig daily. It was noticed that ferric chloride prepared free from copper was as effective as iron plus copper salts in preventing anemia, however the opportunities for copper contamination should not be ruled out. The results of this work, briefly stated, were to show that dietary iron deficiency was of paramount importance among the causes of swine anemia, and that copper deficiency, while theoretically an important factor, was not likely to be encountered under practical conditions.

It is interesting at this point to speculate upon the nature of species differences with respect to prevalence of nutritional anemia in the newborn. Why should the presence of anemia be so common in swine, as compared to other animals, particularly when the swine in question are consuming rations apparently adequate for the demands of reproduction and lactation? One theory which immediately comes to

mind is that the multiple births in swine exhaust the iron reserves of the mother - consequently the young are born with deficient iron stores. These deficiencies could under natural conditions be remedied through supplies available in the soil to the young pigs. In support of this theory, it may be stated that anemia among human children is more prevalent in twins than in single births (112). It seems safe to assume however, that if simple physical capabilities are the basic issue, the relationship between weight of young at birth to the weight of the mother should roughly approximate the incidence of anemia. Some representative fetal and maternal weights for different species are listed in Table 1.

Table 1: Fetal and Maternal Weights for Different Species.

Species	Wt. of mother	Wt. of young	Proportion Young/Mother %	Reference
Cattle	379.0 Kg.	35.9 Kg.	9.4%	42
	495.5 Kg.	41.8 Kg.	8.4%	"
Dogs	16.86 Kg.	1.560 Kg.(5)*	9.2%	150
	14.50 Kg.	0.280 Kg.(1)	1.9%	"
Rabbits	5.9 Kg.	0.096 Kg.(2)	1.6%	211
Rats	317.0 gm.	56.0 gm.(10)	17.7%	this paper
	294.0 gm.	56.1 gm.(8)	19.1%	"
Sheep	68.6 Kg.	3.33 Kg.	4.8%	204
	51.8 Kg.	3.99 Kg.	7.7%	"
Swine	286.5 Kg.	18.25 Kg.(12)	6.4%	this paper
	200.0 Kg.	8.77 Kg.(8)	4.4%	"

* Figures in brackets indicate number of young born.

These data (Table 1) may perhaps be criticized because of the paucity of samples from which they were drawn; however such observations are not numerous in the literature. As far as possible, data of a diverse nature have been included for the different species, viz.: large

and small maternal weights, and large and small litters where the species gives rise to multiple births. The figures do serve to illustrate the point desired, that is that far from having the highest offspring : maternal weight ratio, which might indicate a tendency towards anemia by reason of inadequate iron stores in the newborn, swine actually show one of the lower proportions listed. Conversely, some of the higher offspring : maternal proportions recorded - in the dairy cow and the dog - are in species in which anemia is seldom if ever a problem. The case of the rat deserves special mention, for here the writer's own observations indicate that the litter average 18.4 per cent of the maternal weight at birth, and Alt (4) puts the figure even higher, at 20 per cent. Certainly, if the metabolism of the iron is of comparable efficiency in rats and in swine, one might reasonably expect the development of iron-deficiency anemia in young rats shortly after birth, yet in the writer's experience such is not the case. An investigation of the possibility of using the rat as a test animal for the study of iron-deficiency anemia as encountered in swine was made, and will be discussed later in this thesis.

Mention has been made in the literature (125, pp.537-538) of a polycythemia followed by "physiologic hemolysis" in the newborn, and possibly some discussion of these conditions may prove valuable with reference to the changing blood picture in young swine. It has been often noted that young animals at birth have high hemoglobin and erythrocyte levels, which some investigators attribute to a polycythemia. Various reasons may be forwarded for this condition. Sachs (162)

believes the polycythemia may be caused by traumatic shock at birth, and points out that it is not evidenced in human Caesarean sections. Wintrobe, (205) on the other hand, points out that the newborn at birth closely resembles the fetus in its physiology, and suggests that red corpuscles are produced in excess because of previous oxygen unsaturation in utero. If such is indeed the case, then the rapid decrease in hemoglobin and erythrocyte concentrations after birth might be due to destruction of these immature fragile cells. Considerable controversy exists regarding the mechanisms of these early changes in blood composition, however two significant points have emerged. Young animals of various species are consistently born with elevated hemoglobin and erythrocyte levels, even in the face of nutritional anemia in the maternal organism (180). Just as consistently, these blood components become reduced, often sharply and extensively, by a physiologic anemia during the first weeks of post-natal life. Observations on these reductions have been recorded by Forkner (59) for humans, and by Radeff (157) for various species of domestic animals. It appears therefore that a process of accelerated blood destruction is a common and apparently natural occurrence among the young of higher animals. The problem remains why spontaneous recovery can be effected with relative ease in some animals, while only with considerable difficulty in others, such as swine. Almost certainly the supply of iron to the young is an important factor in this recovery, in which case two items should come in for consideration: the iron stores in the young at birth, and the quantity of iron which may be supplied through the diet shortly after birth. A matter of some importance to this latter item

is the so-called "physiologic age" of different animals at birth, which determines at least in part, the dietary pattern for those animals in the first days of post-natal life.

The concept of "physiologic age" is useful in determining the relative stages of maturity reached by different species at birth. As Brody (18) has expressed it, "Physiologic time is indicated by the tempo of changes in living organisms, and is not proportional to chronologic time as measured by the movement of the hand of a clock adjusted to the rate of the earth's rotationaround the sun." In other words, to compare the blood picture, or any other phase of metabolism, of different species at birth is not physiologically accurate, since they may spend vastly different proportions of their total life span in utero. Reference to Brody's table (19, p.736) shows that the young of the cow and the ewe at birth are physiologically equivalent to the young of the sow and the laboratory rat at 2 to 3 times their birth age, as counted from conception. It is evident then that these latter species are born in a relatively immature condition, and one may infer that they will be more dependent upon their mothers for nourishment than will the species first named.

Since the more immature animals at birth, such as the pig, must of necessity derive their sustenance at first solely from the maternal milk, it may be well to examine comparatively some figures relative to the composition of milk of different species. Such data are presented in Table 2. Components other than iron have been included, after the manner of Abderhalden (2) to show that sow's milk compares favourably with that of other animals in these respects.

Table 2: Partial Composition of the Milk of Different Species

Species	Fat %	Protein %	Lactose %	Ca mg%	P mg%	Fe mg%	Reference
Cattle	3.4	3.2	4.6	106	88	0.072	(69)
Dogs	8.3	7.5	3.7	289	240	?	(5)
Rats	15.0	12.0	2.8	349	270	0.70	(29)
Swine	6.8	6.2	5.0	252	151	0.18	(109,193)

Here at last is some tangible difference between the rat and the pig which may aid in the understanding of the lower incidence of anemia in the young of the former animals. It will be noticed that the iron content of rat milk is over 3.5 times that reported for sow's milk, and it seems possible that the young rats might be able to obtain appreciable amounts of iron through their mother's milk during the nursing period. Unfortunately figures for total milk yield of the lactating rat are not available, and thus a total quantitative comparison of the milk iron secreted by the rat and the sow is not possible. Hughes and Hart in their paper (109) however, estimate total milk production for a sow over an eight-week lactation as 413.2 pounds. If calculations are based on an average litter of 8 young, one might assume that some 0.75 mg. of iron would be available to each pig daily. These figures are only approximations, as Hughes has indicated that the yield of milk per sow varies within rather wide limits. The low incidence of anemia in calves, which receive milk containing only one-tenth the amount of iron as rats can probably be explained by the greater maturity of the former animals at birth, as previously discussed, and to the fact that they are likely able to supplement their dietary iron from sources other than milk at an early age.

To assess the adequacy of the amounts of iron supplied in the sow's milk for the prevention of anemia in young pigs, it is necessary to know the concentration of iron in the young animals when born, and their rate of growth thereafter. As young pigs are apparently born with sufficient iron for a satisfactory blood picture, it seems logical to assume that if the concentration of the metal in the body can be maintained at the birth level, no anemia related to iron deficiency should ensue. Venn and his associates (193) have calculated that a piglet contains approximately 50 mg. of iron at birth, and observe that it should retain about 7 mg. of iron daily in the first few weeks of life to grow normally, without becoming anemic. It may be readily seen that, accepting the validity of the foregoing figures, the young pigs cannot derive sufficient iron from the sow's milk under normal conditions of confinement to prevent the onset of some degree of anemia.

Two main methods of attack suggest themselves for the remedy of this situation. The first method is preventative in nature, and deals with increasing the iron available to the young pigs by way of the maternal organism : either by increasing the concentration of iron in their bodies at birth, or by increasing the amount supplied to them post-partum in the milk. Sporadic attempts have been made at the investigation of these possibilities, however as they are intimately concerned with the metabolism of iron, it seems wise to leave their consideration until the discussion of some phases of iron metabolism, which follows shortly. The second method, and one which has received

widespread attention and practical application, is curative in nature, and involves the supplementation of the diet of young pigs with some form of iron during the nursing period.

Therapy of Nutritional Anemia in Pigs.

Since, as already noted, many of the earlier observations on nutritional anemia in pigs indicated that access to the soil played a large part in alleviating the disorder, it was perhaps natural that supplying soil to young pigs confined indoors should be one of the first methods of treatment attempted. In this connection an interesting characteristic of behaviour of young pigs should be mentioned, namely that although they will not "pick at the trough" and take supplementary feed until some 4 weeks of age, they will display a tendency to root and eat dirt at about the third or fourth day (114). While preliminary attempts (114) at the alleviation of anemia in young pigs through the use of soil followed the rather extreme practice of covering the entire floors of the farrowing pens with soil to a depth of 2-3 inches, later work showed that placing a small shallow box of soil in the pens was sufficient. Doyle (39) was one of the first to report that either blue grass sod or rich soil without the sod kept in the farrowing pen would give "full protection" against anemia. Since then various other workers (52,53,68,93,114,148) have confirmed Doyle's observations on the efficacy of the soil treatment. Provision of soil in the pens has not proved universally effective in cure of anemia. "Soil" of course is a term embracing a very wide selection of

substances, and the efficiency of such substances in anemia therapy varies according to their physical and chemical characteristics. Fargo (52) at Wisconsin, for instance, has reported that finely divided soils like clay loam seem more effective than coarser sandy types, possibly because young pigs tend to consume more of the former. Moe, Craft, and Thompson (141,142) also noted that an appreciable number of their experimental animals were not cured of anemia through access to soil in their pens, and they developed the practice of supplementing 50 pounds of soil with 9 grams of ferrous sulphate and 1.5 grams of copper sulphate,with which method they had considerable success. Venn, McCance, and Widdowson, in one of their papers (193) calculated that the soil used in their experiments contained 1.5 per cent of iron. On analysis of the contents of a young pig's digestive tract, they found a total of 513 mg. of iron, most of which must have come from the soil. It would appear that considerable quantities of iron may be made accessible to pigs through the soil. Unfortunately data regarding the availability of such iron, in terms of the amount absorbed by the animals, are not immediately available. Although the use of soil in control of piglet anemia has proved successful in a number of cases, one further rather important disadvantage to the method should be mentioned. Soil is an excellent medium for the growth of many parasitic organisms, and the contamination of farrowing pens with soil near the critical parturition period is certainly not in accord with many approved sanitation procedures, including the widely-hailed "McLean county system."

Actual iron therapy has been resorted to in many instances

for the control of anemia in young pigs, using various quantities and types of iron salts. Like the soil treatment previously outlined, many of these methods have been empirical in nature, aimed primarily at providing a practical solution to the anemia problem. Several basic factors are involved in this type of therapy. The prime consideration being to supply iron to the pigs, the actual concentration of iron in the preparation used becomes important. For example, ferrous sulphate or commercial "copperas" is cheap and readily available, however it contains only some 20 per cent by weight of iron. Reduced iron on the other hand contains from 90-96 per cent of metallic iron(182). Nor is concentration of the metal the whole story, for the final criterion must be the ease of absorption of the metal through the intestinal wall, in other words the net availability to the animal. More will be said relitive to this point in the discussion of the metabolism of iron. Another factor worthy of consideration is the astringent property ascribed to many iron salts. Some English workers (57) have observed that the use of ferrous sulphate solution has led to constipation unless the dose was carefully regulated. Elvehjem et al have recommended the use of iron pyrophosphate, which is not astringent (49). Of the rather numerous therapeutic methods listed in the literature, a few which have apparently enjoyed considerable successful usage have been gathered in Table 3. In this table the methods cited have been compared with respect to the amount of iron actually supplied to the young pigs. Other methods exist wherein an iron solution is painted on the udder of the sow at nursing, but it is practically impossible to make an accurate quantitative assessment of the iron supply in such cases.

Table 3: A Comparison of Some Methods of Iron Therapy.

Treatment	Estimated Iron Supplied per pig per day.	Reference.
1. 1 tsp. daily of 2.5% sol'n. $FeSO_4$ from 1 week to weaning.	0.07 gm.	3
2. 2 ml. 12% iron phrophosphate sol'n. daily	0.03 gm.	57
3. Ferric citrate (with copper sulphate)	0.025 gm.	91
4. a.Ferric chloride (by capsule: 41 mg. daily)	0.025 gm.	98
b.Ferric oxide (by capsule: 125 mg. daily)	0.067 gm.	98
5. Reduced iron, 0.210 gm. weekly, as single dose	0.03 gm.	41
6. Reduced iron, single large dose of 0.5-1.0 gm., at 1 week of age	0.009-0.02 gm.*	58
7. a.Ferric ammonium citrate 2 gm. daily	0.33-0.37 gm.	115
b.Ferric potassium tartrate 2 gm. daily	0.24-0.30 gm.	115

* based on 6 wk. period to weaning.

These calculations indicate that it is common to supply 25 to 70 mg. of iron daily to young pigs from birth or shortly thereafter to weaning. If one accepts Venn's (193) figure of 7 mg. of iron daily as the retention necessary to maintain a satisfactory blood picture in young pigs, it is evident that the iron preparations used must be in the neighbourhood of 10-28 per cent available to the animals.

The efficiency of the methods of iron therapy outlined in Table 3 for the treatment of anemia of young pigs under practical

conditions has been taken by some to constitute an argument against the further need for research in this problem. It should be recognized, however, that the individual handling of young pigs which is necessary if they are to be dosed with iron-containing solutions daily introduces problems of labour utilization, especially in large operations. Moreover, it should be emphasized that these methods are curative, rather than preventative in nature, and as Schofield (166) has put it, "The ideal way of correcting a deficiency disease in the suckling animal is through the mother, and not in the medication of the young."

THE METABOLISM OF IRON.

Any study concerning the possibility of increasing the iron supplies of the newborn through pre-natal supplementation of the diet of the mother is of course inseparably bound with an understanding of the mechanism of the metabolism of iron. Over the years a voluminous literature has been built up concerning the processes of iron metabolism, but where, for instance, such processes have been rather clearly defined for other minerals, in the case of iron they often remain enigmatic. In the form of radioactive isotopes of iron, the modern physiologist is possessed of tools which hold great promise for the unravelling of some of these mysteries. In cognizance of this fact, material relating to recent investigations making use of radioactive iron has been included in the brief discussion to follow. Indeed, it is seldom that such a double incentive for solution of problems of basic research and practical agricultural production is offered the investigator as in this instance of the implications of iron in anemia of suckling pigs.

Knowledge of the exact metabolic mechanism of iron has grown slowly, for several reasons. While there is a practically universal distribution of iron in biological materials, such iron is quantitatively minute when compared with the extremely prevalent inorganic iron abounding in nature. Accordingly, investigators delving into the iron content of biological materials have always been confronted with a serious problem of contamination from non-biological sources. Furthermore, even within biological materials an inequality of distribution of iron is present, as approximately three-fifths of the iron in an animal body occurs in the form of blood hemoglobin. As blood continually bathes the various body tissues, the problem of determining certain minute fractions of the total iron (such as stored reserves) becomes quite complex.

Absorption of Iron

Bunge and his school(22,pp.370-385) about the end of the last century were among the first to demonstrate that iron can be absorbed in various forms from the digestive tracts of animals. Bunge believed however, and his teachings had no small effect on early thinking in biochemistry, that "food iron" was necessary for the actual construction of the hemoglobin molecule, while inorganic iron might act as a stimulant to, though not as an integral part in, hemoglobin synthesis. It is only in comparatively recent years that it has been recognized that inorganic iron is actually more satisfactory therapeutically than iron in organic combination, or so-called "natural" forms. As a matter of fact, the iron in blood itself is relatively unavailable for

absorption when administered orally to animals. The difference in efficiency between inorganic and organic forms of iron is not wholly qualitative however, and Josephs(110) logically suggests that a great deal of the added efficiency of the former over the latter is due to the higher concentrations of inorganic iron offered. For instance, ferrous sulphate hydrate contains some 20 per cent of iron by weight, while hemoglobin which may be considered a reasonably iron-rich organic compound, contains only 0.335 per cent of the metal.

The main obsorption of iron is apparently from that portion of the duodenum immediately adjacent to the pylorus, (173, p.209) and in fact it was held for some time that there was little or no absorption elsewhere. Recently however, Hahn and his associates, (87) using a dog with an isolated stomach and anastomosis of the esophagus to the duodenum, showed that tagged iron was absorbed through the isolated gastric mucosa as extensively as if there had been an intact gastrointestinal tract. Although this experiment did not take place under normal physiological conditions, the possibility of absorption of significant amounts of iron from the stomach should not be ignored. Evidence of iron absorption from both the small and large intestine in the rat was demonstrated by Copp and Greenberg (81) using Fe^{55}, and these examples may represent species differences in the site of absorption. Acidity undoubtedly plays an important role in rendering food iron soluble. Ferric salts will slowly precipitate out of solution as colloidal ferric hydroxide at $_{p}H$ values above 2.5, while ferrous salts precipitate out as ferrous hydroxide over $_{p}H$ 5. Such hydrogen ion activity would seem to preclude the possibility of iron absorption from

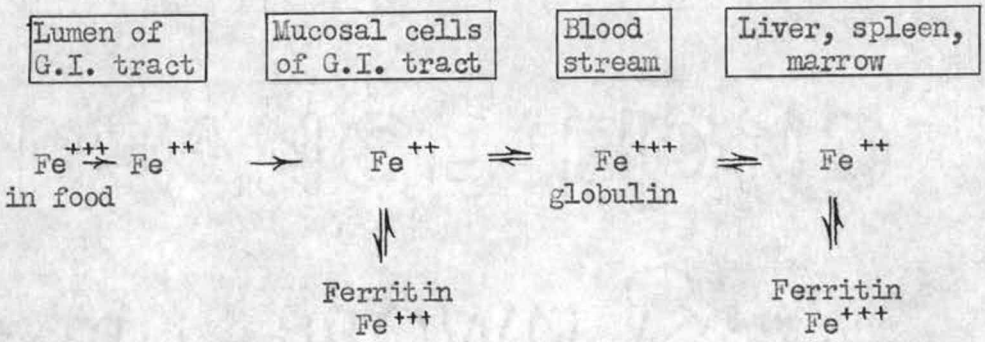
the lower parts of the intestine, since the alkalinity of the bile and pancreatic juices would probably render the metal insoluble beyond the ampulla of Vater (81). An interesting sidelight on the effect of acidity on iron absorption is provided by the fact that many cases of nutritional anemia in humans are accompanied by achlorhydria or gastric anacidity (173, p.209).

A difference of opinion exists regarding the most readily available (i.e. the most easily absorbed) form and oxidation state of iron. Considerable evidence has been built up in experiments on human subjects favouring the ferrous over the ferric form (34,88,89,127,147, 207). It is generally accepted that iron must be in the ferrous state at time of absorption, but it is a moot question whether greater absorptive efficiency may be obtained through supplying ferrous iron in the diet or through a natural conversion of the metal into the divalent state in the acid medium of the stomach. In this regard species differences may again be apparent, for although dietary ferrous iron has been advantageous in humans, there seems to be little difference in degree of absorption of the two forms in dogs and in rats (147,181,191, 201).

The nature of the control mechanisms by which the animal body regulates iron absorption has long been one of the most fascinating phases in the metabolism of iron. Several investigations, notably those of Hahn and his associates (84) have put forward the theory that iron absorption is governed to a large extent by the state of the iron reserves of the body. If the body stores of iron are low, absorption of the metal from the degestive tract becomes increased, and vice versa.

Various workers have demonstrated that the ability of the body to excrete iron is extremely limited, (85,128,200) and it has therefore been postulated that selective absorption may be a protective mechanism which guards against the accumulation of excessive amounts of iron. A process of simple diffusion as the absorptive mechanism for iron is not upheld by the concentration exhibited in the intestinal lumen and the plasma, and the possibility of a labile pool or temporary storehouse for the metal was broached (77). This concept was strengthened when it was noticed that there was an appreciable time lapse between depletion of body stores of iron, (as by bleeding) and an increased rate of uptake of the metal from the gastro-intestinal tract. Granick and Michaelis in a series of interesting experiments (73,74,137) have identified the temporary store of iron as ferritin, a remarkable protein complex which is present in the intestinal mucosa, and which may combine stoiochiometrically with as much as 23 per cent of its weight of iron. Apparently a series of equilibria are involved. The cells of the intestinal mucosa take up iron from the lumen of the gastro-intestinal tract until the stoiochiometric limit of ferritin is reached. When such a condition arises, no more iron absorption is possible until the iron of the plasma globulin, which is in equilibrium with stored iron of the liver, becomes lowered, due to an increased demand. Hahn (81) believes that the reason the process does not take place in reverse is the tenacity with which the plasma iron is bound to the globulin. Granick's scheme for the explanation of iron absorption is portrayed in Figure 3.

Figure 3: Mechanism of Iron Absorption



-after Granick (75)

The efficiency of iron absorption appears, on the whole to be rather low, at least insofar as the concentrations of the metal likely to be found under natural conditions are concerned. While absorptive efficiency is dependent upon the state of iron reserves in the body, it is also regulated to some extent by the amount of iron available in the diet. In parallel with metabolic processes concerning other nutrients, it seems that the organism is able to husband its dietary iron rather carefully if it is in short supply, or to deal much more extravagantly with it if it is plentiful. A range of iron uptake from 60 per cent of a single dose of 12 mg. of radioiron down to only 3.2 per cent of a dose of 115 mg. was demonstrated by Hahn, et al, with anemic dogs (86). It would appear that the efficiency of absorption of single doses of iron of about the magnitude that might be encountered in foods is in the neighbourhood of 28.5 per cent (90). This figure was arrived upon as the mean of 750 observations concerning doses of 2-18 mg. of iron to human pregnancy cases. Comment should be made upon this observation to the effect that the iron administration took

place during the later stages of pregnancy when it might be expected that the insistent demands of the fetus would have depleted maternal stores of iron to the point where absorption would be accelerated.

Extensive data have been presented to support the thesis that the rate of iron absorption is increased when the body's stores of the metal are lowered. Hahn, Bale, Lawrence and Whipple, (84) for example, demonstrated by comparisons of blood iron concentrations that dogs made anemic by bleeding could absorb iron rapidly and efficiently. Chapin and Ross (27) demonstrated with human subjects that where 1 mg. of radioiron per kilogram of body weight was administered, normal absorption was less than 1 per cent, but in cases of iron deficiency anemia absorption rose to 3 to 10 per cent of the amount given. Anemia per se is not, however, the limiting factor in iron absorption. Balfour, (9) using a radioactive isotope of iron, proved that absorption of the isotope by a dog : first in a normal condition, and a few hours later made severely anemic by bleeding, was essentially the same. On the other hand, following bleeding, after the animal had been allowed to approach a nearly normal hemoglobin level once again at the expense of its bodily stores of iron, the absorption efficiency increaded five-fold. Even in the growing child, where one might expect a constant drain on the iron reserves, favouring absorption, the efficiency of iron absorption is only approximately 12 per cent (33). The correct evaluation of efficiency of iron absorption is of course extremely important therapeutically, and lack of such accurate knowledge has been the cause of considerable disappointment and confusion in the treatment of iron deficiency anemias in the past.

The use of a single massive dose of iron for the alleviation of nutritional anemia certainly has much to commend it from the standpoint of practical application. It has generally been found however, that the efficiency of absorption of iron at high dosage levels has been notoriously low. Whipple and Robscheit-Robbins (201) demonstrated several years ago that whereas iron could be absorbed by an anemic dog at some 35 per cent efficiency when dosed at a 40 mg. level, a tenfold increase in the dosage to 400 mg. daily reduced the percentage uptake to 5 to 6 per cent. This means that a tenfold increase in the iron administered merely raised the amount actually absorbed by a factor of two. It would seem therefore that the continued administration of reasonably low levels of iron would be preferable, from the point of view of metabolic efficiency, to the use of larger amounts at less frequent intervals.

Rate, as well as extent, of absorption of iron has been studied through the employment of tracer techniques, with encouraging results. Austoni and Greenberg (8) contrasted the rate of absorption of Fe^{59} (fed by stomach tube in solution as ferric chloride) by two groups of rats, described as normal, and iron deficient. On the average it required about 12 hours for a single dose of radioiron to pass completely from the stomach and the small intestine. Time of passage through the gastro-intestinal tract proved significantly longer in the anemic than in the normal rats, besides which the anemic animals eliminated less of the administered iron in both feces and urine than did the normal ones.

Up to this point the mechanism of iron absorption has been

considered simply insofar as iron itself is concerned, however the influence of other, non-ferrous, materials in the diet should not be ignored. One relationship which became the subject for early study was that between iron and calcium in the diet. Initially, Sherman (171) in 1907 suggested that increased iron absorption might result from the inclusion of larger than normal amounts of calcium in the diet. More recently Rose and Vahlteich (159) and Kletzien (120) have presented evidence for an antagonism between these two elements. These latter investigations demonstrated lower hemoglobin readings in rats supplemented with iron in the presence of excess calcium than with iron alone, and a reduction of the iron content of all tissues except the spleen following the addition of 1 per cent calcium carbonate to the diet. Kletzien suggests that dietary supplementation with a readily ionizable form of calcium provokes an ionic imbalance with consequent abnormalities of behaviour at the protoplasmic boundaries, possibly involving adsorptive effects and pH changes. The question of the effect upon iron absorption of the common agricultural practice of increasing the dietary supplementation of ground limestone and bone meal for farm animals late in pregnancy is certainly one for speculation.

An excess of phosphorous in the diet, similarly to an excess of calcium, will apparently result in a lowered level of iron absorption (37). Shelling and Josephs (170) have explained this phenomenon by stating that a high phosphorous diet should cause the precipitation and excretion of iron in the phosphate form, however their experimental data are rather preliminary in nature. It appears probable that some of the non-antagonistic results reported for calcium in iron absorption

(cf. Sherman, 171) might be attributed to the calcium-binding effect of the phosphorous content of the experimental rations. Again, the agricultural implications, where cereal grain rations naturally high in phosphorous are concerned, are obvious.

Among the organic compounds likely to be encountered under natural dietary conditions, phytates have been questioned by some as interfering factors in iron absorption. Sharpe, et al (169) demonstrated a marked decrease in iron absorption, as measured by use for hemoglobin production, in the presence of sodium phytate. A continuation of these investigations however indicated that naturally occurring phytates as in oatmeal did not appreciably alter the extent of iron absorption any more than did phytate-free milk in the ration.

Various vitamins have been named as having some influence on the processes of hemopoiesis (125, pp.613-617) including riboflavin, pyridoxine, nicotinic acid, pantothenic acid, "folic" acid, ascorbic acid, and vitamin D. The knowledge concerning the mode of action of these vitamins in this regard is, as yet, far from complete; however it appears probable that most of them are involved rather with the non-ferrous portion of the hemoglobin molecule than with the iron moiety. It seems reasonable to assume that ascorbic acid, by virtue of its reducing powers, may have some direct effect on the absorption of iron (132). Heilmeyer and Plötner (125, p.614) demonstrated that injection of iron ascorbate caused a greater increase in hemoglobin formation than might be accounted for by its iron content alone, which suggests that the action of ascorbic acid might be extended to include mobilization of the iron reserves. In case such mobilization took place in

in the intestinal mucosa, iron absorption would be indirectly affected. It should be mentioned that other workers (11,36) have taken a different view, and have attempted to explain increased hemoglobin production in the presence of ascorbic acid by an antagonistic action between ascorbic acid and cobalt.

In cases of pyridoxine deficiency an anemia results which is characterized by high plasma iron, and deposition of hemosiderin in the bone marrow, liver, and spleen (26,135,206). Apparently some inconsistency occurs between the rate of iron absorption and the rate of hemoglobin synthesis, which may be overcome by dietary supplementation with pyridoxine. Vitamin D has been reported to facilitate the absorption of iron from food, likely through some procedure involving its known influence on the metabolism of calcium and phosphorous (37,65,66,67). An interesting speculation on the influence of vitamin D is afforded in the work of Foster (60) who demonstrated a definite increase in the hemoglobin content in the blood of anemic rats subjected to quartz-mercury arc lamp irradiation. This finding is in contrast to the negative effects of irradiation upon anemia reported earlier in this thesis (68,124,136) and Fowler believed the response might be due to "...stimulated activity, bringing about an improvement in the general nutritional state." It may be argued however that this general improvement must have some metabolic cause, which might conceivably involve an iron-vitamin D interrelationship.

A natural sequel to the investigations dealing with the mechanism of iron absorption lies in attempts to provide some technical means of evaluating the efficiency of absorption of iron in different

combinations, including those occurring in natural foodstuffs. Since iron is absorbed in the ferrous form it occurred to some workers that measurement of the amount of iron present in the ferrous form, as by the di-pyridyl reaction, might give an indication of the efficiency of absorption that might be expected (49,50,108,172). The results of such studies however, apparently indicate a degree of availability of iron greater than that actually enjoyed by the animal body, even in an anemic state. Whipple and Robscheit-Robbins (201) in their experiments on dogs made anemic by bleeding showed that iron salts are absorbed and utilized to an extent governed rather by the amount of the metal present than by its valency state at time of ingestion. Heath's statement (105) seems appropriate in summary of the efforts to assess the adequacy of iron in foods: "...of this we can be quite sure: a diet deficient in iron has not been known to produce iron deficiency, except in the presence of increased needs for iron, such as growth, pregnancy, or blood loss."

Iron Transport and Utilization

Following the absorption of dietary iron into the cells of the intestinal mucosa, various mechanisms of transportation and biosynthesis come into play, through which the iron is converted to its form of ultimate utilization. While it is convenient to group the processes of absorption, transportation, storage and excretion under different headings for purposes of discussion, such grouping should be made only with the realization that these processes are actually

successive phases of a continuous operation. According to Granick, (75) whose views have received wide acceptance, ferrous iron moves from the mucosa into the blood stream where it is immediately autoxidized to ferric hydroxide. The ferric hydroxide is then adsorbed onto the serum proteins and is transported as a ferric hydroxide-protein complex (178). It is generally agreed that the blood plasma is the vehicle by which iron is transported in the animal body, and a depletion of the so-called labile reserves of iron has been reflected in a lower value for plasma iron (144,145,146). Normal values for plasma iron have been cited as between 0.1 and 0.2 mg. per 100 ml. of blood, which values, although physically minute are physiologically important as they represent the most labile portion of the metabolic iron (81). Attempts have been made to estimate the quantitative absorption of iron from the gastro-intestinal tract by means of the blood plasma levels. Hahn, (83) however, has pointed out the difficulty of such estimations. Two separate processes are involved, namely absorption through the intestinal mucosa and removal from the blood stream by the hemopoietic system, and these processes, while simultaneous, may occur at vastly different rates.

In consideration of the utilization of iron it seems apropos to cite a table given by Hahn (82) which lists the distribution of iron in different forms in the animal body. This material is reproduced as Table 4 below:

Table 4: Distribution of Iron in Perfused Tissues of the Dog.

Classification	Iron (mg.)	% of total body iron.
Blood hemoglobin iron	900 mg.	57%
Muscle hemoglobin iron	110	7
Total hemoglobin iron	1010	64
Parenchyma iron (muscle and other tissues)	240	16
Available visceral storage (liver, spleen and marrow)	225	15
Available iron (other tissues, estimated)	75	5
Totals	1550 mg.	100%

(after Hahn, 82, p.249)

The preponderance of hemoglobin iron over other forms is at once apparent. It has not been demonstrated as yet whether muscle hemoglobin (myoglobin) iron is involved in an equilibrium with blood hemoglobin and the various storage depots for iron. Quantitative data such as those in Table 4 also serve to emphasize the undesirability of experimental studies of the iron content of unperfused tissues, containing blood which may account for a concentration of iron far greater than that actually held in the tissue itself.

Utilization of iron, insofar as it contributes to the picture of nutritional anemia under study, is reflected in the state of the blood hemoglobin and the stores from which that hemoglobin may be built. While absorption and utilization are intimately related, as previously mentioned, there are certain points of difference between the two procedures which justify their separate consideration. For in instance, while it has been observed that absorptive efficiency is low

when excess amounts of iron are present, there is some indication that utilization of the absorbed metal may be improved in the presence of excess. Fowler and Barer (63) for example, have demonstrated a more rapid increase in blood hemoglobin following the use of excessive doses of iron than with smaller, although apparently adequate, amounts. This finding would suggest a stimulatory action of iron on hemoglobin formation in addition to its action as a replacement therapy.

As was shown to be the case in the absorption of iron, certain non-ferrous constituents of the diet may have a very considerable influence on the efficiency of iron utilization. Notable among such substances is copper, which has been widely investigated in connection with hemopoiesis since the work of Hart, et al, at Wisconsin (97,198, 199). Elvehjem and Sherman (48) demonstrated that addition of ferric chloride to milk diets of anemic rats increased the amounts of stored iron, but did not yield a proportionate increase in hemoglobin production. When copper was added, however, hemoglobin was observed to be formed at the expense of stored iron in the liver, which would suggest that copper plays a role in the utilization, rather than the assimilation, of iron. It seems probable that copper is concerned in some way in the freeing of iron from tissue combination, or in the conversion of labile stored iron into a form suitable for inclusion in the hemoglobin molecule. Elvehjem in his review (43) suggests that copper's role in iron metabolism may perhaps be explained through its effect on the activity of certain enzyme systems. There is some evidence to suggest that the site of action of copper is the iron storage depots of the liver (32,111). It should be mentioned, that, in spite of the

obvious importance of copper in iron metabolism, under practical conditions sufficient copper is nearly always present in the body stores, in the diet, or as a contaminant of therapeutic iron to supply the needs (130, p.275).

With more direct reference to pigs, it has been suggested that hemopoiesis cannot take place rapidly in these animals unless the copper content of their whole blood is maintained above 20 γ per cent (167). Whole blood copper values for pigs have fallen to as low as 7 γ per cent under experimental conditions, which indicates that supply of copper might become a limiting factor in hemoglobin production under rather drastic environmental conditions. Chronic iron-deficiency anemia in swine does not apparently result in a lowering of the plasma copper levels (25) although there is a slight reduction in pyridoxine deficiency anemia (24).

An effect of a totally different nature upon the mechanism of hemoglobin production has been attributed to another metal, cobalt. The addition of small quantities of this metal to the diet of animals has been found to cause a polycythemia believed by Orten (153) to be due to a vasodilation in the region of the bone marrow, with a consequent local anoxemia. Wisconsin workers (177) have estimated that the presence of 0.04-0.05 mg. of cobalt in the entire body of a rat is sufficient to produce a decided polycythemia. Barron (11) and Davis (36) suggest alternatively that cobalt acts rather upon immature red cells, interfering with their normal maturation, and causing their premature release into the circulation. Obscure though the exact mechanism may

be, it seems quite certain that cobalt causes some abnormality in the production of erythrocytes, and this this metal should be considered, at least indirectly, as a contributor in the utilization of iron.

For some time the presence of manganese was given some significance in the production of hemoglobin, and consequently in the utilization of iron, (187) however more recent studies (123) tend to discount such activity, at least insofar as any effectiveness in the cure of nutritional anemia is concerned.

Iron Storage

Wide acceptance has been given the concept that iron is carefully husbanded by the animal body, and is used again and again in the production of hemoglobin. This efficiency of retention, together with the apparent influence of stored reserves of iron upon absorption of the metal from the gastro-intestinal tract, make iron storage a matter of interest and importance. Certain major storage depots for iron have been noted, including the liver, spleen, bone marrow, and the kidneys, (16,131,149,158,188,189) however the widespread presence of iron throughout the animal body suggests that other less-obvious deposits may exist elsewhere. As has already been suggested, the term "storage" need not have any static connotation; in fact certain investigators are of the opinion that excess circulating hemoglobin may itself be considered in the nature of a very labile store (129). The data presented on iron storage are not consistent, due mainly to difficulties in obtaining normal organisms for study,(especially among the higher animals and man) and to the use by many workers of unperfused material.

Among the sites of storage mentioned, the liver is usually considered the most useful as far as influence upon blood formation through dietary supplementation is concerned. There appears to be a fairly constant liver-iron concentration throughout life, according to species, however the total iron may be lowered shortly after birth, presumably due to the low iron intake from milk (126). The spleen has been reported as most active in supplying readily available iron, but the exact status of its iron stores is difficult to arrive upon, due to the amount of iron in the red cells contained in the sinusoids (82). Moreover, the stored iron in the spleen appears to be derived chiefly from hemoglobin through the breakdown of aged red cells, in contrast to that in the liver, which may come more directly from dietary sources.

The efficiency of storage of iron is apparently of a very high order, which re-emphasizes the importance of absorption as the process regulating the state of iron metabolism in the body. It is of course difficult to demonstrate experimentally the actual proportion of iron absorbed through the intestinal wall that is stored, however the work of Fowler and Barer (62) seems to indicate that the storage organs are able to cope with almost any amount of iron absorbed. As absorption of iron is dependent on the extent of stored reserves, it is equally true that the stores are regulated by the body's need. If then, iron is fed to an animal suffering from blood loss, the extent of iron storage will be low, simply because the dose will be almost quantitatively used for hemoglobin production shortly after administration. Hahn (83) has attempted to gauge the extent of normal iron stores in the dog, and has estimated that they are sufficient to allow

for a 30 per cent replacement of blood hemoglobin in time of necessity.

Iron Excretion

Excretion of iron, in the sense of removal of the metal by the kidneys, has long been realized to be of a very low order, and this fact has led to a rather common overstatement that iron excretion is negligible. Actually of course, as Moore points out,(143) iron is a component of every cell of the animal body, and as such must continually be excreted to some extent in desquamation from the tissues. Moore and his co-workers (143) have demonstrated that the main pathway for iron excretion is the feces, where in the human it may amount to 0.3 to 0.5 mg. daily. The separation and identification of fecal iron is difficult, as it may represent unabsorbed dietary iron, desquamation of the epithelium of the gastro-intestinal tract, iron from some secretion, such as bile, or active intestinal excretion. Use of radioactive isotopes of iron holds promise for future elucidation of the precise nature of iron excretion.

McCance and Widdowson (128) performed an interesting experiment to demonstrate that renal excretion of iron could take place to a considerable extent under certain conditions. Producing a hemolytic anemia through the use of phenylhydrazine, these workers were able to show a degree of renal excretion. In the light of this work they suggested that iron excretion by the kidneys is by no means impossible, but is simply by-passed in the normal animal due to the efficiency of the storage organs in keeping the plasma iron content low.

Among the divisions of fecal iron mentioned, it has been

estimated by some investigators that iron lost in bile may amount to approximately 0.2 mg. daily in the dog (103) and in the rat,(76) however these studies were made by biliary fistulae, which would not allow for the possibility of re-absorption. As mentioned above, the total amount of fecal iron is very low, and it seems possible that it might arise totally from desquamated epithelial cells of the intestinal mucosa, involving their ferritin content. In addition to fecal excretion some iron is undoubtedly lost to the animal through sweat and desquamation from the exterior surfaces of the body. Moore (143) has put iron loss through all such other means at about 0.5 mg. daily in the human. Even though the actual loss of iron is small, it is an over-simplification to say that the animal body has no capacity to excrete iron.

In the study of quantitative requirements for various nutrients, the "balance" type of experiment is often employed, wherein an equilibrium is attempted between ingesta and excreta with reference to the substance concerned. In the case of iron metabolism the balance type of study is not entirely valid, because of the difficulty in distinguishing between absorbed and unabsorbed iron in the feces, and because retention is not necessarily a true index of requirements. As has been explained in the foregoing discussion, little of the absorbed iron is excreted, and the amount retained may not be the actual amount needed for immediate utilization (164). Balance trials are of value however, inasmuch as they indicate the relative retention, and hence availability for absorption, of iron from different sources.

Iron in Pregnancy : in Fetus and Newborn

The brief summary on the mechanism of iron metabolism immediately preceding has been presented as a necessary prelude to the study of iron supply to newborn animals, which is the main objective of this work. Several reasons may be given for the method of approach taken, namely that of attempting to raise the iron content of the newborn through supplementation of the maternal diet throughout pregnancy. Iron therapy in human pregnancies is an accepted practice, and it seems possible that it may be successfully adapted to pregnancies in domestic animals. One important point of difference should be noted: in humans such treatment has the purpose of preventing anemia in the mother - in animals it would be undertaken to prevent the onset of anemia in the newborn. Attempts at iron supplementation of the diets of sows through the gestation period have been made in the past, however they have usually consisted of addition of iron in very small quantities (98). In consideration of the low absorptive efficiency generally exhibited for iron, it may be justifiable to repeat such work using a higher dosage level. Finally, there is a possibility of error in the application of some of the data on quantitative iron storage, due to the use in some cases of unperfused tissue, and the failure to separate actual storage reserves from circulating hemoglobin iron. There are two main subdivisions of the subject of iron metabolism in pregnancy: first, the extent and rate of iron transmission from mother to fetus, and second, the most propitious time, in terms of efficiency of uptake, for iron supplementation of the maternal diet.

Much of the information presently available regarding transmission of iron during pregnancy has of necessity been gathered through the use of artificial radioactive isotopes. Although in their own words, "the precise method of transfer of iron from mother to fetus still awaits demonstration", Pommerenke et al (155) have made some interesting observations regarding placental transmission of iron in humans. Using radioactive iron, they demonstrated presence of the isotope in the fetal circulation within 40 minutes of the time of administration of the substance to the mother. Considering the fact that it requires some 5 hours before presence of radioiron may usually be shown in the red cells of an animal, (138) it is evident that there must be some remarkably efficient method of transfer between mother and fetus.

Many years ago, Haecker (80) at the University of Minnesota made the statement that, "in order to determine the actual net nutrients required to produce a given animal product, ...the composition of the product should be known." It seems permissible to classify the young of animals in Haecker's category of "animal products," in which case analyses of the feti should give some indication of the extent of supplementation of the mother's diet which should be practised. Mitchell and his associates (139) have followed this line of reasoning in their very painstaking and comprehensive study of the requirements of pregnancy in swine. These investigators sacrificed pregnant sows at different stages of gestation, and made analyses of the contents of the uteri. It was of course impossible to follow any one litter throughout gestation, but composit figures could be estimated. The estimate was made that a total of 581 mg. of iron would be required

for the total composition of the products of conception of a litter of 8 young pigs throughout pregnancy. In comparison, Wohl, (208, p.458) states that the average human fetus at birth contains 246 mg. of iron, in addition to which the mother has been observed to store some 550 mg. during pregnancy. How closely this latter figure might be applied to other species is a question. At any rate, assuming an efficiency of absorption of iron under the stress of pregnancy of 28.5 per cent, (124) Mitchell's figure of 581 mg. would represent a requirement of 2,038 mg. iron in the diet. This figure divided by the length of the gestation period for the sow, which is 114 days, would indicate that a daily supply of 18 mg. of iron should be sufficient to meet the needs of an average litter. Provision of such a small amount of iron in the diet is an easy matter, however the fact remains that this rate of supplementation is not sufficient to prevent the young from the onset of anemia. It seems that either the magnitude of the requirements has been underestimated, which is unlikely, or else allowance has not been made for a sufficient degree of wastage in the absorption of the iron supplied. The question that remains to be answered is whether addition of amounts of iron considerably in excess of the calculated requirements to the diet of the pregnant sow will have any marked effect on raising the iron reserves of the young. Successful use of rather high dosages of iron in human pregnancy - in amounts up to one gram daily, (71, p.1113) - suggests the possibility of favourable results elsewhere.

One item which should come in for serious consideration in connection with iron supplementation during pregnancy is the time of

optimum utilization. In other words, should iron be administered with the diet in equivalent amounts daily throughout the gestation period, or should it be graduated in some way so as to coincide with a period of most efficient utilization? It is to be expected that if the magnitude of iron requirement follows the extent of weight increase in the fetus, the time of greatest necessity for iron supply to the mother would be towards the latter end of gestation. Some experimental findings with regard to iron uptake by human feti have tended to confirm this concept. Hahn demonstrated (81,p.308) that uptake of radioiron by pregnant women reached a maximum efficiency during the last 10 weeks of pregnancy, and that that maximum was almost double the efficiency exhibited in the first 20 weeks. Swanson and Iob (184) have calculated that iron retention by the human fetus in the last two months of pregnancy represents 67 per cent of the total iron content of the fetus at term. The last two months of a human pregnancy is equivalent to the last 25 days of gestation in swine, and a transposition of Mitchell's figures (139) indicates that pig feti accumulate only 35 per cent of the term content of iron in this latter phase of gestation. Apparently then there is a species difference in the rate of iron transfer from the maternal to the fetal organisms, which may have a bearing on later prevalence of anemia. Probably more important in the eventual occurrence of iron deficiency anemia however is the early rate of post-natal growth in the different species: for example a doubling of birth weight occurs in about three months in humans, as compared with seven days in pigs.

Unfortunately, the whole picture of intra-uterine transfer of iron has only been partially investigated, and some conflicting data

have appeared in the literature. Gladstone (70) has reported no evidence, either chemically or microscopically, for any large or progressive depositions of iron in the liver of the human fetus during the last four months of pre-natal life. Such findings should probably be interpreted as meaning that most of the iron retained by the fetus is either utilized as circulating hemoglobin, or stored in some labile form. The nature and extent of liver stores of iron in the newborn has come in for considerable comment. Lintzell et al (cited by Venn, 193) listed analytical data from piglets at birth which suggest that the liver store of iron is of a magnitude which might make it useful as a reserve for hemoglobin production. On the other hand, Fontès and Thivolle (55,56) demonstrated a considerable increase in the body iron content of puppies during suckling, and calculated that the increase was far greater than could be supplied by liver stores alone. These workers suggested that a considerable proportion of iron required by young animals after birth may be supplied through the milk, and only when the milk is insufficient are the liver reserves of iron brought into use. The experiences of these French investigators emphasize the danger of attempting to use iron metabolism data gathered with one species of animal in application to another species, without modification. Schmey (165) reported in 1899 that the iron content of fetal swine liver at term was 26.06 mg. per 100 grams, as compared to 21.23 mg. per 100 grams at maturity (on a wet weight basis), which is indicative of a storage condition in the young. Needham cites findings from investigations with the bovine species which are closely parallel - indicating an elevated iron concentration in fetal liver (152,

p.1277). The actual amount of iron in the livers of newborn pigs has been estimated by Mitchell to be around 0.018 per cent, (139) and by Lintzell (79) to total about 8 mg., therefore it would seem that these reserves would not be able to play any very prominent role in hemoglobin production. These figures were taken from apparently normal animals born to mothers on an unsupplemented diet, and whether it would be possible to raise such stores markedly through addition of iron to the diet in pregnancy remains a question.

Mention should be made in passing of Barcroft's studies dealing with hemoglobin formation in sheep (10, p.89). In common with other investigators, Barcroft believes that however great may be the rate of formation of hemoglobin in the feti during the first half of the gestation period, the drain on the iron reserves of the mother should be practically microscopic in extent. Pursuing the point further, however, he notes that the quantity of hemoglobin in the circulation of the ewe dropped almost one-quarter during the same portion of pregnancy. Towards the end of gestation, on the other hand, when the feti are accumulating hemoglobin at a remarkable rate, the concentration of hemoglobin in the blood of the ewe remains fairly constant. Barcroft included only three sheep in his observations, however his experiments were of such a precise nature as to warrant consideration. While it is difficult to correlate the blood picture of mother and fetus in the face of these apparent anomalies, it would appear from this work that the early part of pregnancy should not be overlooked in scheduling the most opportune time for iron supplementation of the diet.

In summarizing the data presented from the literature

relative to iron metabolism and anemia of pigs, it must be admitted that in many instances the processes involved are far from clear. Certain trends in thinking on the subject are apparent, however, and with respect to these it is possible to list some salient points. The anemia of young pigs has been shown to be predominantly one of iron deficiency, and one which will respond to iron therapy. The young animals are born with apparently normal hemoglobin levels and with some (rather small) stores of iron in the liver. The mother exhibits a normal hemoglobin concentration throughout gestation, but this is not apparently indicative of a satisfactory blood condition in the young shortly after birth. Such a comparison between mother and young is in contrast to that which occurs in humans, where moderate anemia may develop in the mother, although the young may be normal through the critical early post-natal period. Attempts to raise the iron available to young pigs through addition of iron to the diet of the sow both before and after parturition have not generally been successful, although the amounts of iron employed have been small. The milk of the sow has been shown to be lower in iron content than that of some other animals which give rise to multiple births, such as the rat, and one may conclude that young pigs are victims of circumstance. They have been so fitted by nature as to be most susceptible to anemia unless they are fortunate enough to be raised under "natural" environmental conditions in which they are able to supplement their diet with iron from the soil.

Several points which may be of value have been brought forward in the course of the discussion on iron metabolism. It has been pointed out that of all the multifarious processes making up the

metabolic scheme for iron, absorption is the dominant one. Once taken into the animal body, iron is easily and efficiently utilized: it is the absorptive mechanisms which regulate the tempo of iron utilization. Iron absorption is itself adjusted according to the need exhibited for iron, which means that it is at its most efficient during times of metabolic stress such as rapid growth, or pregnancy. Confusion exists as to the most available form of iron for therapeutic use, because species differences apparently exist. Broadly speaking, the preparation used should contain a fairly high proportion of iron which is capable of being freed by the hydrochloric acid of the stomach; it should be reasonably palatable and not extremely astringent. Imbalance of other mineral elements in the diet will interfere with the absorption of iron in some cases, however it may be reasonably assumed that if the non-ferrous elements are balanced to meet the general requirements of the animal they will not interfere with the iron uptake. Quantitatively, iron is used most efficiently when the supply is somewhat restricted, however iron salts are not generally expensive, and the possibility of economical use of massive dosage levels should not be ignored. Copper has been shown to be a necessary adjunct to iron in hemoglobin synthesis, however the quantities of copper necessary are so minute that it seldom becomes a limiting factor under practical conditions. Some scattered work has been reviewed concerning the phenomena of iron transfer from the mother to the fetus during pregnancy. Again controversy is apparent, as species differences have been demonstrated. Certainly results have not been put forward which would

categorically deny the possibility of raising the iron content of young pigs through adequate administration of iron salts to the sow throughout pregnancy. In some of the experiments which follow, attempts are described to effect such iron transfer to the point where iron-deficiency anemia will not occur.

EXPERIMENTAL INVESTIGATIONS.

INTRODUCTION

In initiating experiments relative to iron-deficiency anemia in swine, it has seemed logical to proceed first with the actual establishment of the condition in untreated animals. In other words, a base level for control observations must be made, for it is obviously impossible to demonstrate a curative procedure on animals unless they are definitely experiencing the disorder in question. In the experiments which follow, therefore, some relevant data pertaining to the occurrence of anemia in young pigs raised in confinement are presented at the outset. A difficulty which was noted in the preparation of the review of literature, and again in the planning of actual experiments was that different methods of determination of hemoglobin concentrations were employed by different workers. Accordingly, after a method was decided upon for use in this work, it was standardized with respect to the actual iron content of the blood. These initial experiments did little, in themselves, to aid in the prevention of baby pig anemia, however it was felt that they were a necessary foundation which had to be completed before more extensive investigations could be undertaken.

Experimental work with the larger species of domestic animals is desirable because it yields results which may be applied directly in practice on the animals in question. Such work also has disadvantages however, possibly the most serious of which is the time involved. In

order to follow the effects of any specific treatment through pregnancy and perhaps the early stages of growth in pigs requires that at least four months elapse before the results may become known. If smaller laboratory animals, such as rats, could be used, on the other hand, a comparable physiologic period could be covered in a matter of a single month. The advantages of such a saving in time were obvious, especially in the preliminary phases of experimentation, and it was resolved to attempt to use the rat as an experimental subject, and determine whether an analagous type of anemia might be produced in this animal.

With regard to the actual approach to the objective of this thesis: that is the investigation of the effect upon baby pig anemia of iron supplementation of the sow throughout pregnancy, a wide range of possible experiments might be suggested. As has been brought out in the literature survey, there are several questions to be answered, including the relative efficiencies of different forms and oxidation states of iron for the pig, the most efficient level of administration and the time of administration at which to secure the most efficient transfer of iron from the mother to the feti. Any of these are worthy of investigation, and might be developed into full-scale projects. Limitations of experimental facilities and of time and space, however, necessitated the narrowing down of investigations to more restricted lines. It was decided, and it is hoped with justification, to limit the choice of iron preparations used to two which have enjoyed successful use in therapy under other circumstances: one with pigs and one with humans. Reports in the literature appear to favour continued administration of iron over a considerable period rather than use of a

single massive dose for optimum uptake, and there is some evidence which indicates that iron therapy should be practised in the early as well as the later stages of pregnancy. Accordingly the experiments have been devised to allow for continuous supplementation of the diet with iron throughout pregnancy, and, in view of the many interfering factors in iron absorption, at a higher level than that practised in any similar work in the past.

EXPERIMENT 1. Demonstration of Iron-Deficiency Anemia in Young Pigs Born in Confinement in the Oregon State College Herd: Blood Hemoglobin Levels and Liver Iron.

Numerous investigators already mentioned in the literature review (40,41,115,133,154,168) have reported on the blood picture of young pigs suffering from iron-deficiency anemia. The consensus of opinion is that a hemoglobin concentration of 6 grams or less per 100 ml. of blood is indicative of this anemia, (115) however the values may go much lower. Erythrocyte counts, although not perhaps as useful as the hemoglobin determinations, have nevertheless been reported as contributory evidence for the presence of anemia. It was determined to assess the blood picture of some untreated young pigs in the Oregon State College herd in order to determine their value for anemia-prevention experiments. As the liver has been named as one of the major storage sites for iron in the animal body, (193) it seemed that coincidental determination of liver iron might be useful.

Method

For hemoglobin determinations, 5 ml. samples of blood were drawn, citrated, and subjected to analysis using a Spencer Hemoglobinometer, model 1000. (American Optical Co.) Erythrocyte counts were made on separate samples, by the method of Kolmer and Boerner, (122, pp.57-61) using Hayem's diluting fluid. The method of obtaining the sample and preparing it for reading is described in more detail in Appendix A. The animals for investigation were simply taken at random

from the College herd, with the one distinction that some had access to the soil shortly after birth, while others were confined to concrete-floored pens. The sows in both cases had outdoor runs, with pasture, throughout the gestation period, but received no iron supplement in their rations. While it was thought highly desirable that an examination be made of the early mortality cases in the herd, it was of course impossible to perform an accurate hemoglobin analysis post-mortem. A gross examination was performed in such cases however, and the livers were removed for analysis. Classification of these animals was made into two groups: those which were stillborn, and those which died within the first week of birth. Two animals of comparable age which were apparently in good health were also sacrificed and their livers analyzed to serve as a norm.

The iron determinations on liver tissue were carried out according to a modification of the methods of Bruckmann and Zondek, (20) Kennedy, (113) and Thompson, (186) described more fully in Appendix B. A note should be inserted here relative to the preparation of the samples, since it is in this phase of analysis that confusion has usually occurred in the literature. In assessing the extent of iron storage it is necessary to effect a separation of reserve iron from iron which is in active use, as in circulating hemoglobin. It might be argued of course, as Fontès and Thivolle have maintained, (55) that excess hemoglobin might act as a reserve, but if this is so there is no practical method of separating that excess and of estimating its extent. Various methods do exist for the separation of hemin and non-hemin iron in the tissues, including perfusion, washing, and extraction with

specific complex-forming agents. Perfusion of tissues is probably preferable, since it leaves the tissue intact, however it is not particularly effective when carried out some time post-mortem, when the blood has clotted. For this reason perfusion was not resorted to in this work. Washing with distilled water might be practised to remove hemoglobin iron, but it is questionable whether, if the tissue were macerated to ensure complete removal of hemoglobin, some of the non-hemin iron might not also be carried away. Tompsett (189) who has done considerable research on the iron content of biological materials, prefers a specific extraction agent, such as sodium pyrophosphate, followed by precipitation of the hemin iron by trichloracetic acid. It was decided that this latter procedure would be suitable for the work in hand, and it has been followed throughout in the liver analyses reported later. The method employed in the actual sampling of the livers is described in Appendix A.

Results and Discussion

As the work of others has suggested that the minimum blood hemoglobin level in young pigs is usually reached between two to four weeks, (see Figure 1) the samples reported on here were drawn at an average age of 18 days. Results of hemoglobin assay and erythrocyte count are presented in Table 5.

Table 5: Hemoglobin Levels and Erythrocyte Counts for Pigs 18 Days After Birth

Animals kept in Confinement		Animals with Access to Soil	
Hemoglobin gm./100 ml.	Erythrocytes million/cu.mm.	Hemoglobin gm./100 ml.	Erythrocytes million/cu.mm.
5.5	3.8	6.8	5.0
6.5	4.2	7.3	5.8
6.0	4.2	7.0	5.0
5.2	4.5	8.2	6.2
5.8	4.0	8.4	6.4
5.4	6.0	8.0	6.2
7.0	5.8	7.5	6.0
5.9	4.2	6.9	5.2
6.5	4.2	8.8	6.5
5.0	4.0	7.4	6.0
Ave.5.9	4.5	7.6	5.8

It is evident that higher hemoglobin levels and higher erythrocyte concentrations are present in the animals which had access to the soil, as might be expected. The hemoglobin levels of the pigs maintained under conditions of confinement were higher than many reported in the literature, and averaged only slightly under the generally accepted "anemic level". This discrepancy may perhaps be explained on the basis that the sows to which the above animals were born all had access to pasture during pregnancy. If such explanation is valid, then it offers some encouragement for the possibility of improvement of the early blood picture of the young through pre-natal dietary supplementation.

The results of the liver analyses run for non-hemin iron in the different groups of pigs are presented in Table 6.

Table 6: Comparison of Iron Stores (non-hemin iron) in the Livers of Young Pigs

A. Apparently normal animals sacrificed for analysis:

Body Wt.	Liver Wt.	Non-hemin Iron in Liver		
gm.	gm.	Total mg.	%wet wt.	%dry wt.
1180	26.48	1.58	.0062	.0201
1096	22.70	1.02	.0045	.0164
Average		1.30	.0053	.0182

B. Pigs born alive, but died within 12 hours:

Body Wt.	Liver Wt.	Non-hemin Iron in Liver		
gm.	gm.	Total mg.	%wet wt.	%dry wt.
999	21.50	1.46	.0068	.0222
817	18.37	1.23	.0067	.0202
1089	24.02	1.95	.0082	.0349
863	17.57	1.38	.0079	.0368
636	17.08	1.52	.0089	.0334
953	23.42	1.67	.0071	.0272
635	19.04	2.30	.0121	.0301
726	21.03	2.40	.0115	.0450
772	19.70	1.96	.0100	.0322
816	21.83	2.28	.0100	.0430
590	14.48	0.87	.0061	.0221
1000	27.06	3.02	.0112	.0419
409	9.48	0.86	.0090	.0314
1362	26.12	0.72	.0028	.0150
1453	31.19	1.15	.0037	.0175
Average		1.65	.0081	.0302

C. Stillborn pigs:

Body Wt.	Liver Wt.	Non-hemin Iron in Liver		
gm.	gm.	Total mg.	%wet wt.	%dry wt.
1452	39.20	2.82	.0072	.0241
1226	37.06	2.37	.0064	.0215
908	29.49	2.60	.0088	.0297
954	32.36	2.33	.0072	.0242
545	12.50	0.90	.0007	.0024
1362	24.20	2.52	.0104	.0352
1370	37.01	1.18	.0032	.0109
Average		2.10	.0048	.0211

It would appear from examination of these figures (Table 6) that there is little difference in the liver iron stores of apparently normal pigs and of those which died shortly after birth. The concentration of iron, judging by the average of the three groups, is actually higher in the livers of the animals which died than in the normal animals. There is virtually no difference in the iron concentration picture between the stillborn animals and those which died after birth, as the larger total stores in the former group are undoubtedly due to the larger liver weights of the animals subjected to analysis in that group. The outstanding feature of these data is the smallness of the iron stores involved, and it is obvious that amounts of the magnitude of one to two milligrams of iron would not be of any great value for hemoglobin production over a long period.

The thought may be advanced that the method of analysis could be at fault, in that it is not accounting for all the non-hemin iron actually present. Reference to the literature indicates good agreement with previously published work. For example, Elvehjem et al report stored iron in pig liver on a dry weight basis within a range of 0.0100 to 0.0314 per cent,(98) and 0.0255 to 0.0265 per cent,(17) while Sherman (172) gives figures for "available iron" on a similar basis, (but determined by the bypyridine method) of 0.0652 per cent. Obviously the most convincing argument for the efficiency of the method is one involving actual recovery of added inorganic iron from liver preparations. Such recovery was satisfactorily accomplished, and the pertinent data are presented in Table 7.

Table 7: Iron Recovery from Liver Tissue
Fe added to sample (as sol'n. of pure iron wire) - 0.010 mg.

	Non-hemin iron in sample mg.	Total non-hemin iron found mg.	Iron recovered mg.	%
	.005	.014	.009	90%
	.015	.025	.010	100%
	.012	.022	.010	100%
Average	.0107	.0203	.0096	96%

It must be admitted that these preliminary data were discouraging at least insofar as they tend to minimize the importance of liver iron stores in the production of hemoglobin in the young pig. Several reasons may be advanced however for the continuation of iron storage investigations. First, all the data reported herein have dealt with animals which have not had the benefit of iron treatment, and there is no guarantee that stored iron cannot be raised to useful levels by dietary supplementation. There is some evidence that the extent of iron storage in the liver can be raised to high levels if there is interference with the processes of iron utilization,(130,p.237) which indicates at least the possibility of increasing depot iron. Further, as the stores of iron are constantly drawn upon, in times of necessity, for hemoglobin production, the iron storage condition should be properly interpreted in conjunction with the blood picture. Second, it is also possible that iron transfer to pig feti during pregnancy may improve their storage of total iron in some form other than that investigated in the liver - possibly through excess hemoglobin storage in the spleen.

EXPERIMENT 2. Standardization of Hemoglobin Determination Procedure.

Much of the difficulty in making an accurate interpretation of the anemia studies on young pigs reported in the literature has been due to the variety of methods used in hemoglobin determinations. There has been a strong tendency in recent years toward reporting hemoglobin levels in grams per 100 ml. of blood rather than as percentages of some arbitrary normal figure. This former method is considered vastly superior in work with swine where the normal figure is subject to rapid and extensive changes during the early weeks of life. Nevertheless, additional standardization seems necessary, due to the difference in mechanism of the various methods available for hemoglobin determination. Kernkamp (116) has made an important contribution in this direction by correlating the results obtainable through use of the Tallquist, Dare, and Newcomer methods. More recently, however, a convenient form of hemoglobinometer has been produced by the American Optical Company, (Spencer, model 1000) and it was decided to standardize this apparatus against actual iron content of the blood used, as determined by Kennedy's method (113).

Method

Preparation of the blood sample for reading in the instrument is the part of the operation where an error is most likely to occur. Some note of explanation of the procedure involved is necessary at this point. The blood is drawn into citrated tubes as outlined in Appendix A, after which samples for analysis are placed in a porcelain spot plate

and hemolysed with saponin. When hemolysis appears complete, a drop of the blood is placed between the two glass plates which make up the observation field of the instrument, and the reading is made. It is obvious that any dilution or concentration of the blood will affect the apparent hemoglobin level, and it was decided to investigate whether evaporation (which occurs when a large number of samples are set out in a spot plate) might noticeably affect the results. To test this possibility, several aliquots from the same original blood sample were set out in a spot plate and read at varying intervals of time. Hemolysis was accomplished by the addition of a knife-point of solid saponin to each blood aliquot, to avoid complications from use of saponin solutions as noted by Ponder (156). Judging from the appearance of the hemolysed blood samples, the grade of saponin used for hemolysis seemed to be a factor of importance in the final hemoglobin determination. Accordingly, comparisons of the hemoglobin readings obtained from aliquots of another blood sample, hemolysed by different samples of saponin available, were made and recorded.

Finally, in order to determine the accuracy of the complete method as described, the iron content of one gram samples of blood was compared with the expected iron content calculated on the basis of the hemoglobin level given by the instrument. Direct analysis has shown the iron content of hemoglobin to be 0.335 per cent,(209) although it may vary slightly from one species to another. Following a standard procedure evolved as a result of the preparatory investigations just described, hemoglobin determinations were made with the Spencer instrument and compared with actual iron analysis data obtained on one gram

samples of the same blood by the Kennedy (113) method.

Results and Discussion

That evaporation does, indeed constitute an interfering factor through concentration of the liquid media of the blood, and consequent elevation of the hemoglobin values, is shown in Table 8.

Table 8: Effect of Evaporation of Blood Samples on Hemoglobin Level Subsequently Determined*

Sample No.	Time of Reading (minutes from start)	Hemoglobin (gm./100ml.)
1	0.00	9.8
2	3.00	9.8
3	6.00	10.0
4	9.00	9.8
5	14.00	10.0
6	18.00	10.3
7	22.00	10.4
8	28.00	10.4

*Temperature at time of reading: 26.9°C
Barometric pressure: 761mm. Hg

From these data it is evident that evaporation may cause higher than actual hemoglobin readings to occur. The procedure was adopted therefore of transporting blood samples for examination as quickly as possible to the laboratory. If readings could not be made immediately, the samples were kept under refrigeration. Prior to reading in the instrument samples were withdrawn from the blood tubes and hemolysed in the spot plate one by one, so that a minimum of exposure was allowed. This procedure was followed throughout.

The use of the glucoside saponin in the hemolysis of blood

has been widely and successfully practised,(117) however this product as sold for chemical purposes occurs in varying degrees of purity. Several saponin samples were available for this study, and their relative efficiencies in hemolysis of blood are listed in Table 9.

Table 9: Effect of Varying Saponin Samples upon Hemoglobin Level Subsequently Determined

Saponin, Lot No.	Source	Hemolysis	Hemoglobin(gm./100ml.)
204	Unknown	Complete	12.4
21109	Merck	Complete	12.5
2930	J.T.Baker	Complete	12.5
33469	Merck	Complete	12.5
21547	J.T.Baker	Incomplete	14.3

Apparently some variation in hemoglobin levels may result from the use of different samples of saponin. The reason for this variation is not immediately apparent, however it would not seem that it is caused by impurities since excellent results were obtained with some of the older, and cruder, lots of saponin. It would also appear from the results in Table 9 that visual evidence of completion of hemolysis (the changing of the blood from its normal opaque appearance to a clear, very dark red solution) is a satisfactory criterion on which to judge the suitability of saponin samples for use in hemoglobin determinations.

The accuracy of the hemoglobinometer used in terms of concentration of hemoglobin read as contrasted with the concentration calculated from the blood iron content determined by direct analysis is shown graphically in Figure 4. It will be observed that in all cases the actual iron found by analysis was lower than the amount calculated to

be present on the basis that iron makes up 0.335 per cent of the hemoglobin molecule. In all, 24 different hogs' blood samples were checked in this way, and showed an average determined iron content of 0.263 mg. compared to an expected iron content of 0.285 mg., which introduces an error of 7.42 per cent. The error appeared to be less in the case of anemic bloods having a low hemoglobin content than in the normal bloods and had an over all variation from 3.69 to 11.85 per cent. In terms of grams of hemoglobin per 100 ml. of blood, an error involving 0.022 mg. of iron in one ml. of blood (the average, above) would represent a difference of 0.6 gram in the instrument reading. While the sensitivity of the instrument would certainly allow for the correction of such an error, the rather wide variation in the errors, plus the small number of samples available for checking, made it seem inadvisable to introduce a correction factor. Consequently, the readings as taken from the Spencer hemoglobinometer have been recorded without any alteration as the hemoglobin levels of the animals involved. The validity of this procedure is borne out by the wide range of hemoglobin concentrations studied: certainly an error of half a gram of hemoglobin would do little to mask the difference between normal figures in excess of nine grams per 100 ml. of blood and those of anemia, which are commonly less than six.

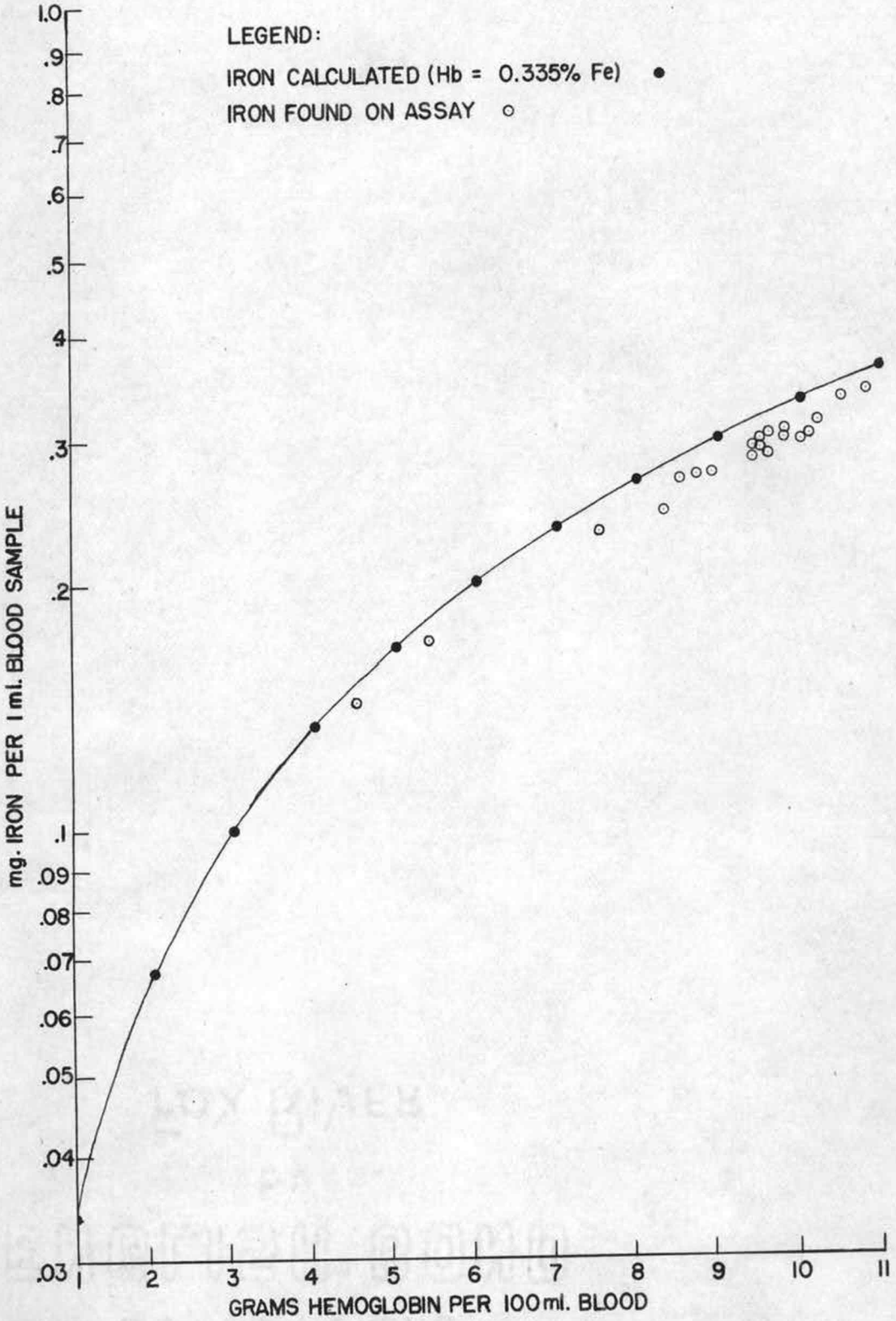


FIGURE 4 : HEMOGLOBINOMETER READINGS COMPARED WITH IRON CONTENTS OF BLOOD

EXPERIMENT 3. The Suitability of Rats as Experimental Animals for the Investigation of Anemia of the Type Occurring in Young Pigs.

Although we are indebted to studies carried out upon laboratory rats for much of the knowledge that has been built up concerning nutritional anemia, it has been illustrated earlier in this thesis that the analogy between experimental anemia in rats and the anemia of young pigs is not clear. The similarities existing in the numbers of young born and the relative rate of growth of those young in these two species of animals is quite striking, and the saving of time which might be accomplished through the use of rats has made investigation of the suitability of these animals seem worth while. Large numbers ob observations on rats receiving normal laboratory diets show some fluctuations in hemoglobin values with age, but no concentrations low enough to be construed as anemia (203). In general, anemia studies with rats have involved the use of animals of a second generation fed a diet deficient in iron, and it has been demonstrated that such animals will develop an anemia during the second week of life or shortly thereafter (4,94). Sometimes anemia can be produced in rats in one generation (47) but significantly it has always occurred after weaning. The following experiment was therefore devised to determine whether, through carefully controlled experimental conditions, young rats could develop a "spontaneous anemia" prior to weaning, similar to that in young pigs.

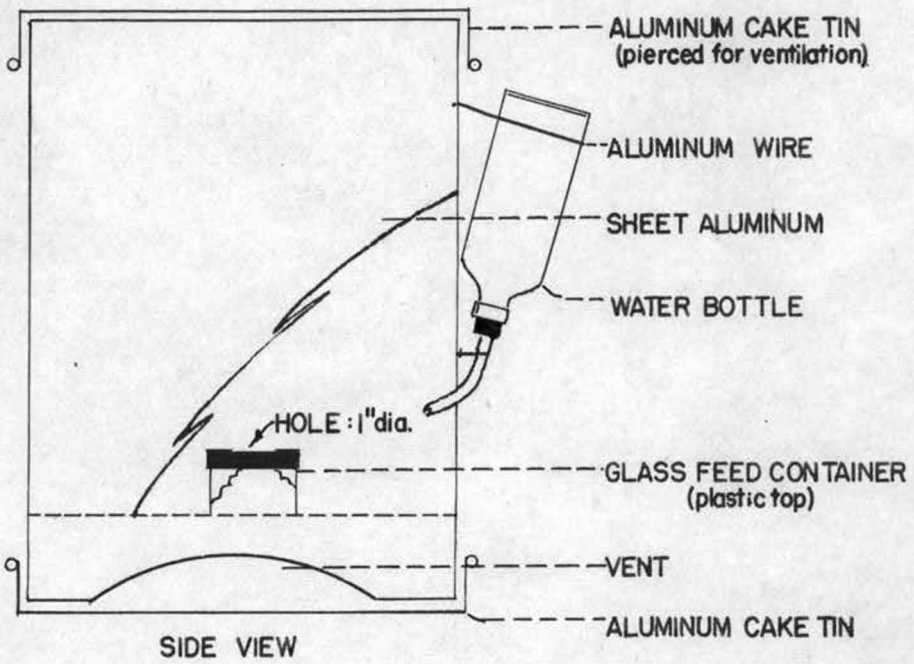
Method

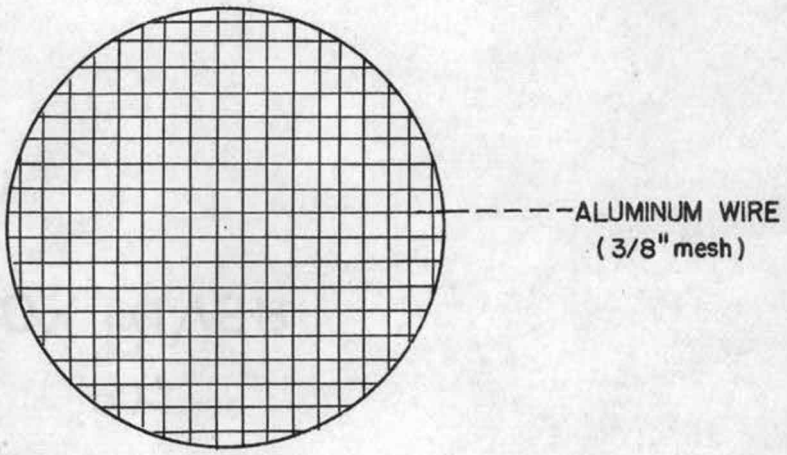
In the use of small experimental animals, like the rat, for study of iron-deficiency anemia, great care must be taken to prevent

access to any iron from non-dietary sources. The possibility of interference in this connection through the use of ferrous metals in cages and food containers has been demonstrated by Miss Mitchell (140). Subsequently the Wisconsin group (174) devised a glass cage floored with glass tubing in an effort to eliminate all possible chance of contamination by inorganic elements. This apparatus proved no more successful however, indeed it actually delayed the onset of anemia as the feces did not pass freely through the floor, and were hence available for coprophagy. In the light of these studies, it was determined to rear the experimental rats in aluminum cages, having floors of aluminum wire mesh through which the feces might pass freely, and with food provided in non-metal containers. The details of the apparatus used are presented in Figure 5.

Female rats were removed to these aluminum cages on the day that they produced their litters, and for the first week cotton batting was provided as bedding for the young. The females were not removed from the cages for feeding, however the constricted openings on the feed containers prevented any visible spillage. The young therefore were maintained throughout the experimental period solely on a diet of their mothers' milk - a procedure which is analagous to the pre-weaning treatment of pigs, during which anemia consistently develops. To further strike a parallel between the environmental conditions available to the rat and the pig, the female rats used in this study were maintained throughout pregnancy on the identical brood sow rations used in the Oregon State College herd. These rations appeared entirely adequate for the rats as the litters produced were of satisfactory size at birth

FIGURE 5: APPARATUS FOR RAT ANEMIA EXPERIMENT





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and grew normally thereafter.

Weaning of young rats usually occurs at about 28 days of age, which is one-half the length of the pre-weaning period in pigs. If the rat was to prove a satisfactory experimental animal for these anemia studies, it would mean that a condition of anemia should be produced in the young prior to 28 days, and if to strictly parallel that in the pig between 7 and 14 days of age. Accordingly, young rats were removed from their mothers at intervals during the pre-weaning period, and blood samples were taken (see Appendix A) for hemoglobin determination. The hemoglobin picture was complemented by analysis of entire young animals for total iron, and the latter figures were compared with examples in the literature. Prior to analysis the gastro-intestinal tracts were slit lengthwise and washed with tap and distilled water to remove all food material. Due to the complication of total iron figures introduced by removal of blood, one-half of each litter was subjected to the hemoglobin determination, and one-half to the iron analysis.

Results and Discussion

The hemoglobin contents of the blood of the young rats concerned in this experiment are given in Table 10, together with comparable values for normal animals from the stock colony. Criticism may be levelled at these data in that they are not continuous with reference to identical animals. It was felt more important to obtain relatively large blood samples for analysis rather than to maintain continuity, particularly since repeated sampling would cause a deviation from the normal, and might induce anemia symptoms not in any way traceable to

the dietary regimen. It is evident from Table 10 that some physiologic lowering of the blood hemoglobin level occurred in the young rats through the pre-weaning period, and that this lowering was slightly pronounced by the use of aluminum cages, however the condition produced is by no means equal in severity to that occurring in young pigs. This fact is illustrated more clearly in Figure 6, and it seems inadvisable to refer to the experimental anemia produced in young rats as a duplicate of that found in suckling pigs.

Table 10: Hemoglobin Concentration in the Blood of Weanling Rats

A. Rats reared in aluminum cages:

Age in Days	No. of Determinations	Ave. Body Weight gm.	Ave. Hemoglobin gm./100ml.
Birth	6	5.6	9.0
7	4	10.1	8.8
10	4	11.8	8.7
17	8	13.4	7.5
21	8	26.2	8.5
28	6	30.2	8.2

B. Stock rats reared in galvanized iron wire cages:

Age in Days	No. of Determinations	Ave. Body Weight gm.	Ave. Hemoglobin gm./100ml.
Birth	4	5.1	9.6
7	2	9.9	9.4
17	2	20.2	8.1
28	6	29.6	8.2

Total iron found in the bodies of the young rats is listed in Table 11. In view of the consistent elevation of the hemoglobin levels it was determined to analyze for iron at three points only during the pre-weaning period, that is, at birth, 7 days, and 28 days.

FIGURE 6: HEMOGLOBIN LEVELS IN YOUNG RATS & YOUNG PIGS

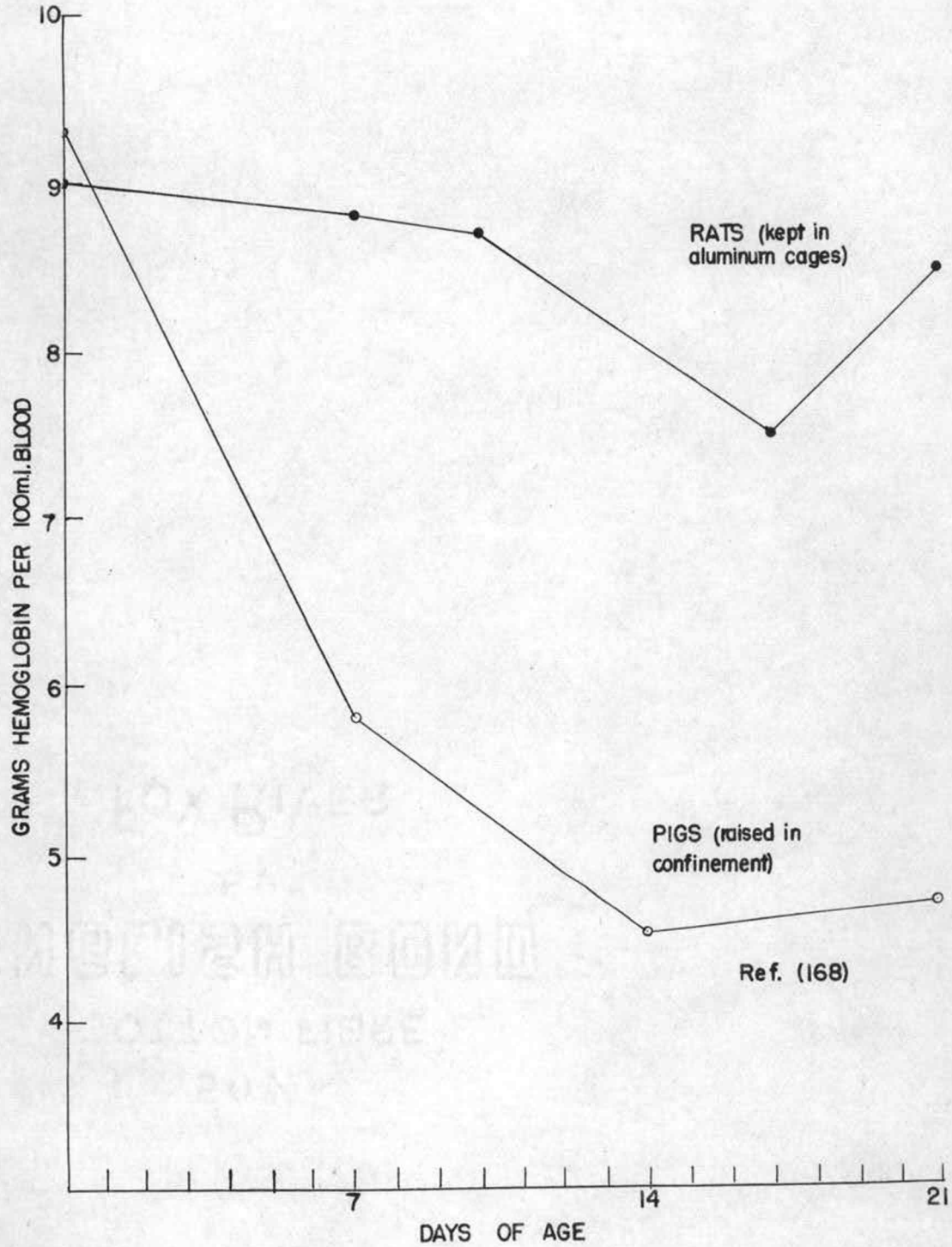

Table 11: Iron Content of Young Rats

Age in Days	No. of determinations	Ave. body weight gm.	Ave. iron found mgm.
Birth	6	5.954	0.369
7	4	10.010	0.381
28	6	25.295	0.745

It will be noticed that the young rats made an average net gain of 0.376 mg. of iron (over double their content at birth) during the nursing period. Since these animals were confined in aluminum cages and had no access to solid food, it may be assumed that these increases came via the milk of the mother. At first glance these data may not seem consistent with the declining hemoglobin values reported, however if calculated as a percentage of body weight it will be seen that iron concentration actually dropped from 0.0062 to 0.0029 per cent.

Smythe and Miller (175) have published data regarding the iron content of apparently normal laboratory rats on stock diets, wherein they cite averages of 0.279 mg. of iron or a concentration of 0.0055 per cent at birth, and 0.806 mg. or 0.0026 per cent at 20 days. The figures reported herein do not appear unreasonably low, in view of the strict environmental conditions imposed.

It has been concluded from the results of this experiment that the laboratory rat is not a suitable animal for the investigation of nutritional anemia of the type prevalent in young pigs. Young rats are apparently able to maintain their blood hemoglobin level above the anemic range even when restricted to a diet of their mother's milk. The reason for this phenomenon may be the fairly high iron content of

rat milk, as suggested in the early part of this thesis.

EXPERIMENT 4. An Attempt to Avert Iron-Deficiency Anemia of Young Pigs through the Feeding of Ferrous Sulphate to the Sow during Pregnancy.

Probably the most widely and successfully used of the various iron salts employed in anemia therapy of young pigs has been ferrous sulphate, or so-called commercial "copperas". This salt lends itself to use in practice because of its cheapness and availability, its solubility, and its fairly high iron content. A disadvantage claimed against it is its astringent action, however this is a common property of many iron compounds, and not generally troublesome through normal dosage levels. Certainly, on the basis of past experiences, there seems as much justification for the use of ferrous sulphate in the cure of piglet anemia as thereis for any other iron preparation. Only scattered attempts have been made toward the supply of additional iron to young pigs by placental transmission brought about through dietary supplementation of the mother. Of these, an early instance (98) recorded negative results which probably discouraged further work along this line for many years, however these results may possibly have been due to the small amounts of iron supplied, (up to 50 mg. daily). More recently some evidence has been brought forward to suggest a benefit from the addition of rather massive amounts of ferrous sulphate to the rations of pregnant sows. Urbanyi (192) in 1941 noted that the regular feeding of iron salts containing some copper to sows during the last one-third of pregnancy prevented anemia and resulted in larger and heavier litters. Urbanyi used dosage levels which supplied 80.8-202.0 mg. of iron, and 8.2-20.5 mg. of copper daily, and he made the interesting comment that the effect on the young pigs was greater when

supplementation was through the mother than when it was direct. In 1949, workers at North Dakota (21) reported higher hemoglobin levels at birth and higher birth weights in young pigs subsequent to supplementation of the sows' rations with copperas at the rate of one-half ounce daily. This latter rate of supplementation seems excessive from the earlier discussion of the iron requirements and efficiency of iron metabolism in pigs, however both these references support the investigation of higher dosage levels of iron than previously attempted.

Method

It was decided to investigate the effect of supplementation of the diet of pregnant sows with iron and copper salts in the proportion of 10 Fe : 1 Cu, as evidenced by the blood picture of the mother and the blood picture and iron storage levels of the young. Two groups of Berkshire sows chosen at random were fed ferrous sulphate ($FeSO_4 \cdot 7H_2O$) and copper sulphate ($CuSO_4 \cdot 5H_2O$) in their daily ration in amounts to supply 100 mg. of iron and 10 mg. of copper per animal. One group was given access to pasture and soil throughout pregnancy, while the other was kept under dry-lot conditions. A control group of animals received the same rations less the iron and copper supplements. In order to ensure that lack of exercise did not become a limiting factor in the performance of those sows denied access to pasture, and in order to comply with the space limitations for housing, this group was composed of three animals only. The other two groups, that is the control, and those receiving supplement and pasture, comprised seven animals each.

The iron and copper salts used were made up in a solution to supply 10 mg. of iron and 1 mg. of copper per ml., and appropriate amounts of the solution were poured over the sows' dry ration daily. This method of supplementation was considered superior to the addition of either dry salts or solution to the feed before mixing, as it minimized the loss prior to feeding. Also as the quantity of the feed intake varied from time to time and from animal to animal, a pre-mix of the supplement with the feed would have necessitated preparation of numerous separate feed mixtures.

Hemoglobin determinations were made upon the sows during pregnancy, to ascertain whether any increase in level might result from the iron supplementation. It was not expected that any condition of anemia would be approached in these mature animals, particularly since those that were not supplemented with iron salts had access to the soil. Hemoglobin determinations and erythrocyte counts were made upon the blood of the young pigs, both at birth and at two weeks of age, and liver iron analyses were made in the cases of the young which died from each group. The methods followed in making these determinations are described in Appendices A and B.

Results and Discussion

Only a very slight difference was noticeable in the blood picture of the sows from the various groups during pregnancy. All groups began the experiment with essentially the same blood hemoglobin level: the control group 10.6 grams, and the supplemented groups with and without pasture 10.8 and 10.65 grams per 100 ml. respectively.

Within the last two weeks of gestation rechecks revealed altered levels amounting to 11.75, 12.35, and 12.3 grams per 100 ml., in the same order. While it has been recognized that anemia in the pregnant sow is rarely apparent, (usually appearing only in cases of flagrant iron deficiency) these figures are interesting in that they demonstrate the possibility of maintaining high hemoglobin levels through iron supplementation without access to soil. It will be noticed that the hemoglobin level in the supplemented group denied pasture is higher than that in the control animals.

The hemoglobin data accumulated from determinations on the blood of the young pigs are presented in Table 12 together with other relevant material.

Table 12: Farrowing Records of Sows and Hemoglobin Levels in the Blood of Young Pigs

Number of sows	Treatment	Young Farrowed Total	Lived	Died	Mortality %	Hemoglobin (gm./100 ml.) Birth	14 days
7	Control	68	54	14	20.6	10.2	8.1
7	Fe,Cu,soil	65	54	11	16.9	10.5	8.4
3	Fe,Cu.	23	18	5	21.7	10.4	8.7

Several points of interest emerge from these data. First, while it is evident that a higher hemoglobin level was attained through iron supplementation of the sows diet, the increase does not appear of great significance, since an anemic level was not reached in the control animals. Further, the mortality, although considerably lower than that reported for animals from this same herd in the introduction to this thesis, was still high enough to suggest the implication of factors other than anemia in baby pig losses. Rather wide variations were

noticed in the hemoglobin levels of different pigs in the same litter, which suggests an unequal distribution of iron from the sow to the young. It seems altogether probable that some of the animals which died may have been on the low end of the iron distribution, which means that the 14 day hemoglobin values among the survivors may be higher than a true litter average. If this condition existed, it would of course be most evident in the group with the highest mortality, that is those animals supplemented with iron and copper but not having access to soil.

Erythrocyte counts were made coincidentally with the hemoglobin determinations, but they appeared to add little to the blood picture as established by the former, and hence were omitted from the tabulations. The numbers of erythrocytes per cu. mm. varied from 7.08 to 7.41 million at birth, and from 4.85 to 5.38 million at about 14 days, and the arrangement among groups was the same as for the hemoglobin values. The compilation of erythrocyte counts, although convenient from the point of view of the small blood samples required, is not as direct an assessment of the blood picture, particularly as related to iron, as is the hemoglobin determination.

Non-hemin iron assays were carried out on samples of liver tissue from those animals which died in each group on experiment. While it is realized that these animals may have been somewhat lower in stored iron than their surviving litter-mates, it is probable that they are indicative of the extent of iron stores as far as their value against anemia is concerned. Neither the blood picture nor post-mortem examination of these animals revealed incidence of anemia. The liver

storage picture is given in Table 13.

Table 13: Liver Iron Storage as Influenced by Pre-Natal Iron Supplementation

Treatment	Animal Weight average	Liver Weight average	Non-Hemin Iron in Liver Total mg.	% *
Control	1566 gm.	33.31 gm.	1.518	.0046
Fe,Cu,soil	1192	27.52	1.830	.0067
Fe,Cu	1115	26.36	1.766	.0067

* % non-hemin iron calculated on wet-weight basis

It would seem from investigation of the non-hemin or stored iron in the livers of the young pigs that although these stores can be increased through placental transfer subsequent to iron supplementation of the sows' diet, the magnitude of such increases is not such as to make them valuable for the prevention of anemia. Indeed, the total liver stores of iron, even under the two supplementation regimes, only averaged 1.798 mg. per animal investigated. Translated into terms of hemoglobin, this iron would represent only slightly over half a gram of the blood pigment which might be added to the system. The significance of half a gram of hemoglobin in the body of a rapidly growing pig must certainly not be very great.

In terms of total iron administered, these amounts stored may be related to approximately 10,000 mg. of iron supplied through the diet of each sow (since administration began about the second week of pregnancy) in addition to any iron obtained from the dietary ingredients or the soil. The litter average of the sows on this experiment was 9 young, whose livers would contain, at the average

figure mentioned above, a total of only slightly over 16 mg. of storage iron. While undoubtedly some of the iron supplied would pass into actively circulating hemoglobin, it would seem that ample excess had been provided for the establishment of considerably higher liver stores. Since no anemia appeared in any of the animals on experiment, one is tempted to believe that iron storage may have been effected elsewhere: possibly in the form of non-active hemoglobin in the spleen. It would be desirable to correlate the iron storage in the young with that in the mother, however the danger of hemorrhage coincident with liver biopsy made liver assays on the sows inadvisable, especially during the latter part of pregnancy.

EXPERIMENT 5. Investigation of Pre-Natal Supplementation of Sows' Diets with Molybdenized Ferrous Sulphate with Respect to the Blood Picture and Liver Iron Stores of the Young

The slow and sometimes incomplete response to existing methods of iron therapy in the treatment of human anemias has provoked more-or-less continuous search for more effective iron preparations. In the course of such investigations, Neary (151) in 1946 explored the efficiency of a complex formed through the co-precipitation of molybdenum sesquioxide (Mo_2O_3) and ferrous sulphate ($FeSO_4 \cdot 7H_2O$). This preparation was administered to pregnant women, who showed somewhat subnormal hemoglobin values, with most encouraging results in terms of hemoglobin regeneration. Comparison of equal quantities (240 mg. of ferrous iron daily) of the molybdenized salt with ferrous sulphate showed a marked advantage in efficiency in favour of the former. Further, at this high dosage level it was found that the objectionable gastro-intestinal side effects produced with ferrous sulphate were absent in the case of the molybdenized complex.

Other investigators have confirmed Neary's initial success with molybdenized ferrous sulphate in treatment of anemia of pregnancy. It was held by some that the poor results from treatment with ferrous sulphate in Neary's experiment were due to too low a dosage level. Healy (104) however, using exsiccated ferrous sulphate to supply 380 mg. of ferrous iron daily, still reported considerably increased hemoglobin formation from molybdenized ferrous sulphate supplying 230 mg. of elemental iron daily. Chesley and Annitto (28) reported mean hemoglobin increases of 2.77 grams per 100 ml. over a six week period with

the molybdenum complex as compared with 1.59 grams with ferrous sulphate. Similarly Dieckmann and Priddle (38) observed almost twofold greater efficiency in terms of mean daily hemoglobin increase in favour of the molybdenized salt. All these investigations were carried out upon human pregnancy cases, and were quite comprehensive. The mechanism of the action of molybdenum in connection with iron has not yet been explained, however one of the writers referred to (104) suggests that it is "...a true example of potentiation of the therapeutic action of iron, which manifestly is brought about either by a possible increased absorption or a more complete utilization of iron." It is difficult to justify the results in terms of the present knowledge of the role played by molybdenum in the animal body, in fact much of the material published in this connection deals with the toxic qualities of excesses of this metal (51,54).

Unfortunately none of the work thus far reported on the efficiency of molybdenized iron in alleviation of anemia of pregnancy has been followed through with reference to the blood condition of the offspring. All of these investigations have been explored solely with respect to the hemoglobin level of the mother, and one is left with the assumption that once this level has been set on a sufficiently high plane, the blood picture of the young will in turn be satisfactory. As previously mentioned, no such assumption is valid in the case of pigs, where a normal maternal hemoglobin level through pregnancy holds little apparent relationship to the hemoglobin concentrations in the young at, and shortly after, birth. In view of the fact that this field is as yet unexplored however, and encouraged by the successful

stimulation of hemoglobin production in human investigations, it has seemed appropriate to conduct experiments involving molybdenized ferrous sulphate with swine.

Method

The approach taken for preliminary investigation has been to supply molybdenized ferrous sulphate[1] to sows during pregnancy, and to assess the result in terms of the blood picture and iron storage in the young. The first problem to occur involved the dosage level to adopt, since no work has been done previously concerning this supplement with swine. One might arbitrarily select some characteristic, such as body weight, on which to base the level of administration. If weight were the criterion, some three times as much of the supplement would have to be used in the case of sows as was employed with women. Such a dosage level for pigs would include some 43 mg. of molybdenum daily (183) - rather a large quantity in a diet comprising three to four kilograms of dry feed, even though absorptive efficiency is probably low (185). In view of what has already been inferred regarding possible toxic qualities of molybdenum, it has seemed wise to restrict the dosage level to 25 ml. of solution daily - approximately that used in successful therapy with women. A dose of 25 ml. would supply 250 mg. of ferrous iron, and 15 mg. of molybdenum.

Three pairs of pregnant Berkshire gilts comprised the groups on experiment. One pair, a negative control, received no iron

[1] ...supplied as "Mol-Iron" liquid, through the courtesy of White Laboratories Inc., Newark, N.J.

supplement of any kind, and was maintained under dry-lot conditions. The second pair received molybdenized ferrous sulphate complex poured upon their grain mixture every second day, in amounts equivalent to 25 ml. per gilt daily. Supplementation commenced the fifth week of pregnancy. The third pair of animals received no iron supplement, but had free access to pasture throughout gestation, thus serving as a normal control, demonstrating effects obtainable under essentially "natural" conditions. The use of gilts in their first pregnancy was decided on because such animals are still growing themselves and hence competing with the feti to some extent for the available iron. It would seem that efficiency of iron absorption should be greatest, while any tendency toward anemia would be most marked, under these conditions.

Observations were made of rate of increase in weight of the gilts during pregnancy, and of numbers and birth weight of the young. Hemoglobin levels were recorded for the mothers during pregnancy, and for the young at birth and 18 days of age, when such values would be expected to be minimal. Non-hemin iron in the livers of the young which died from gilts on the three different treatments was recorded, all techniques followed being exactly similar to those adopted in the previous experiment and described in the Appendices. The non-hemin iron in the liver was investigated in this study because of indications from New Zealand work (130,p.259) that molybdenum feeding coincident with copper deficiency caused considerable iron storage in the liver. Although copper was not deficient in the rations used in this study, the thought occurred that the high rate of molybdenum administration might through antagonistic action reduce the

concentration of copper available to the animals.

Results and Discussion

The maternal growth rates and the farrowing data of the gilts on experiment are presented in Table 14. The animals in each group were allotted purely at random, since considerable variation was noticeable in their initial weights.

Table 14: Maternal Weights and Farrowing Data

Treatment	Ave.wt. of pregnant gilts (pounds) 2 wks.	14 wks.	Young born (ave.)	Ave. birth wt. gm.	Mortality %
No supplement,no soil.	224	316	9	1239	44.4
Mol-Iron supplement.	260	368	6	1253	8.3
No supplement, soil.	249	321	7	1271	21.4

The one striking feature among these data is the differerce in mortality among the young born to females undergoing the different treatments. One pig only died of the twelve born to the gilts receiving molybdenized ferrous sulphate, and that one may well have been accidentally crushed, as it showed none of the characteristic gross symptoms of anemia. It has seemed proper to record this one death in the mortality figures however, in order to keep a true comparison. In spite of the small numbers of animals on experiment, the difference in rate of mortality seems sufficiently great to suggest some metabolic advantage to the young in the molybdenized iron-supplemented group.

Hemoglobin levels recorded on analyses of the maternal blood and that of the young are given in Table 15. It will be noticed that there is essentially no difference in maternal hemoglobin concentrations relating to the various treatments. This may suggest one of two things: either the absorption of molybdenized iron, in common with other iron preparations, varies with the body's need for iron and hence is not great where no anemia is present; or else the results of feeding this material have been manifested rather in the blood picture of the young. It is interesting therefore to note the difference in hemoglobin levels of the various young, especially at the 18 day reading. At this time the hemoglobin concentration in the animals born to the gilts receiving the molybdenum-iron complex was consistently higher than that of the young in the two unsupplemented groups. At birth however the hemoglobin picture was different, and little variation was apparent among the groups. It would seem probable that the young born to the supplemented group were possessed of stores of iron in some form which could be mobilized into hemoglobin where necessary: i.e. when rapid growth increased the blood volume at a rate which could not be matched by the dietary iron supply. Such stores could not logically have existed as excess circulating hemoglobin, or their presence would have been made known at birth. They may, however, have taken the form of relatively stable liver iron, or perhaps even of non-active hemoglobin stored temporarily in the spleen.

Table 15: Hemoglobin Concentrations in the Blood of Mothers and Young

Treatment	Maternal Hemoglobin (gm./100 ml.) 76 days pregnant	Ave. Hemoglobin of Young (gm./100 ml.) Birth	18 days
No supplement, no soil.	11.55	10.55	6.60
Mol-Iron supplement.	11.70	10.70	7.80
No supplement, soil.	11.70	10.63	6.70

The possibility of significant hemoglobin increases being produced from hon-hemin iron stores in the liver seems rather remote after consideration of the data in Table 16. It seems evident also from these data that the molybdenum administered in the molybdenized ferrous sulphate complex has not, even at the high concentrations used, interfered with the action of copper in hemoglobin synthesis. This thought is of course supported by the fact that hemoglobin levels were higher in the young born to the supplemented gilts, as previously shown in Table 15.

Table 16: Non-hemin Iron in Livers of Young as Influenced by Molybdenized Ferrous Sulphate Supplementation of the Diet of the Mother

Treatment	No. of analyses	Ave. animal wt. (gm.)	Ave. liver wt. (gm.)	Non-hemin iron in liver total (mg.)	% *
No supplement, no soil.	2	931	28.39	1.273	.0044
Mol-Iron supplement.	1	817	22.73	1.542	.0067
No supplement, soil.	2	953	26.93	1.797	.0066

* % non-hemin iron calculated on a wet-weight basis

A close similarity is noticeable between the percentage on non-hemin iron stored in the young pig livers subsequent to molybdenized iron supplementation and that recorded in the previous experiment after ferrous sulphate administration. It seems possible that these values (ca. .0067 per cent) represent a maximum figure for this type of iron storage.

The role of molybdenum in iron metabolism has not been made clear through this experiment, however it would appear that the form used, the sesquioxide, is not absorbed through the intestinal wall to any great extent. If it were, one would expect a severe interference with the action of copper in hemoglobin formation, and judging from the increased hemoglobin levels no such interference has occurred. Accordingly, one is tempted to suggest that molybdenum sesquioxide may act catalytically in the gastro-intestinal tract to increase the efficiency of the iron absorption mechanisms. With due regard for the limitations in numbers of animals included in this experiment, it would appear that the use of molybdenized ferrous sulphate in rations of pregnant sows merits further investigation with respect to the prevention of anemia in the young.

SUMMARY OF EXPERIMENTAL OBSERVATIONS

1. Presence of nutritional anemia has been demonstrated through hemoglobin determinations made on young pigs born and maintained under "dry-lot" conditions. The prevalence and severity of such anemia has been shown to be extremely variable, however it appears definitely to be an important contributory cause of early mortality in pigs.

2. A relatively new method for determination of hemoglobin concentrations, employing the Spencer hemoglobinometer, has been investigated and found to be both convenient and accurate for work of this kind. Some experiments have been conducted and recommendations made regarding the treatment of blood samples prior to hemoglobin assay.

3. The laboratory rat has been shown to be unsuitable, even under rigidly controlled environmental conditions, for use as an experimental animal in study of anemia of the baby-pig type. Apparently young rats are able to maintain their body iron at a satisfactory level throughout the nursing period - possibly by virtue of the higher iron concentration in the milk of the rat, as compared to the sow.

4. Supplementation of the diets of pregnant sows with 100 mg. of iron in the form of ferrous sulphate and 10 mg. of copper as copper sulphate daily was found to have only a slight beneficial effect upon the maintenance of satisfactory hemoglobin levels in the young.

5. Administration of 250 mg. of iron as molybdenized ferrous sulphate

daily to sows during pregnancy apparently allowed the maintenance of elevated hemoglobin levels in the young. The action of molybdenum in this regard is not clear, however some suggestions have been made with respect to possible modes of action.

6. The magnitude of non-hemin iron stores in the livers of young pigs makes such stores appear of doubtful value for maintenance of the hemoglobin level.

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APPENDICES

APPENDIX A BIOLOGICAL METHODS

1. Procurement of Blood Samples

a. Young pigs (birth to 4 weeks of age):

The size of the animals involved makes it difficult to obtain satisfactory blood samples by use of a bleeding needle in the ear vessels of the young pigs. If erythrocyte counts only were made, it was found convenient to take a blood sample directly into a dilution pipette at the time of ear notching for identification. In the Oregon State College herd, such identification is made within 24 hours of birth.

Blood samples for hemoglobin determinations were of 2 to 5 ml. volume, and were obtained in two ways. 5 ml. samples could be drawn from the anterior vena cava, using a 1½ inch, 15 gauge needle, and a 10 ml. syringe. Both needle and syringe were rinsed in a saturated solution of sodium citrate before use. The sample was transferred from the syringe to a 10 ml. test tube containing 2 drops of saturated sodium citrate solution, and the contents swirled to ensure thorough mixing. As an alternative, 2 ml. samples of blood could be drawn from an open incision in the tail of the animal, directly into the depressions of a porcelain spot plate containing 1 drop of citrate. If this latter method were followed, of course the hemoglobin determinations would have to be made immediately.

b. Pigs over 4 weeks of age:

Blood samples from larger animals could be easily obtained from the vessels of the ear. In practice, the animals were first restrained by passing a noose around the upper jaw, and snubbing them close to a post. An inspection of the ears for suitable vessels was made, as the ear notching sometimes destroys the marginal veins. The ear surface decided upon was freed of hair with a hand clipper, and a tourniquet of ¼ inch o.d. rubber tubing was applied around the base of the ear. This procedure caused the vessels to dilate markedly, and bleeding was easily accomplished using a 14 gauge bleeding needle rinsed in citrate. 10 ml. blood samples were drawn into test tubes containing 3 drops of saturated citrate solution. At conclusion of sampling the tourniquet was immediately removed, and bleeding soon ceased. This method was considered preferable to the use of toluene or some other vaso-dilatant, in which case bleeding was sometimes difficult to stop.

c. Rats:

The procurement of blood samples of a suitable size from young rats necessitated the sacrifice of the animal. Blood samples were drawn into the depressions of a spot plate, which contained one drop each of saturated sodium citrate, and hemoglobin was determined immediately. Two methods were used effectively for drawing the blood. In the first, the young rat was first anaesthetized

using a cotton inhaling cone soaked in ether. The anterior vena cava was exposed and slashed with the tip of a scalpel, allowing the blood to drain into the spot plate. In the second method the animal was anaesthetized as before, but the blood was drawn by heart puncture, using a 20 gauge needle and a 2 ml. syringe, both rinsed in citrate. After the removal of the blood the animals were given an overdose of ether.

2. Preparation of Blood Samples

a. Erythrocyte count:

For erythrocyte counts, whole blood was drawn directly into a blood dilution pipette to the lower mark, and then diluted to the upper mark with Hayem's blood diluting fluid, composed as below:

Hayem's Blood Diluting Fluid

Distilled water.....200 ml.
NaCl (C.P.)..... 1 gm.
Na_2SO_4 (reagent, crystals)..... 5 gm.
$HgCl_2$..... 0.5 gm.

The method followed for counting was exactly as outlined by Kolmer and Boerner (122, pp. 57-61).

b. Hemoglobin determination:

For hemoglobin determination citrated blood was placed in the depressions of a spot plate and hemolysed with a knife-point of saponin (about 5 mg.). As soon as hemolysis appeared complete, that is when the blood darkened and clarified, a sample was removed on the tip of a finely drawn out piece of glass tubing, and placed between the glass plates provided for reading in the Spencer instrument. One blood sample was followed through completely rather than setting up a number simultaneously, in order to minimize errors caused by evaporation.

3. Preparation of Liver Samples

The method adopted for non-hemin iron determination in liver tissue required small (1 gm.) samples. Appropriate samples were obtained by freezing the liver, and then slicing it very finely, using a bright scalpel blade to minimize contamination. Outer parts of the liver that might have been somewhat dehydrated were discarded, and the samples for analysis were taken from the central part of the organ. Samples sliced thinly in this way were easily broken down in the mortar prior to the addition of the reagents.

APPENDIX B CHEMICAL METHODS

1. Determination of Iron in Whole Blood

Iron in blood was determined in connection with the standardization of the hemoglobinometer. The method used was essentially that of Kennedy (113) for the digestion, and of Thompson (186) for the colorimetry, with some minor modifications.

a. Digestion:

1 ml. of blood is carefully pipetted into a perfectly clean 100 ml. beaker. 10 ml. of a mixture of proportions of 5 ml. conc. H_2SO_4 and 2 ml. 60% $HClO_4$ are added, and the mixture digested on a hot plate. In about 10 minutes the digestion mixture becomes colorless or nearly so, and completion is detected by a rapid ebullition followed by slow boiling and appearance of dense white fumes of SO_3. After cooling, 3 drops of conc. HNO_3 are added to offset any later tendency of the CNS ions to undergo oxidation, reducing the iron. The mixture is diluted to 100 ml. with distilled water.

A similar procedure is carried out on measured amounts of standard iron solutions, thus compensating for any iron in the reagents used and at the same time equalizing the acidity of standard and unknown. In practice, a stock standard was made using 1.727 gm. hydrated ferric ammonium sulphate (M.W. 482.2) and 5 ml. 36N H_2SO_4 per liter. Alternatively, a standard solution could be made by dissolving 0.400 gm. of pure iron wire in 10 ml. 36N H_2SO_4, 10 ml. 6N HCl, and 4 ml. 15N HNO_3, heating the mixture to volatilize excess acids, and diluting to 2 liters. Either stock standard solution supplied 0.2000 mg. Fe per ml. Using dilutions of these stock standards, a standard reference curve was arrived at, as shown in Figure 7.

b. Color Development:

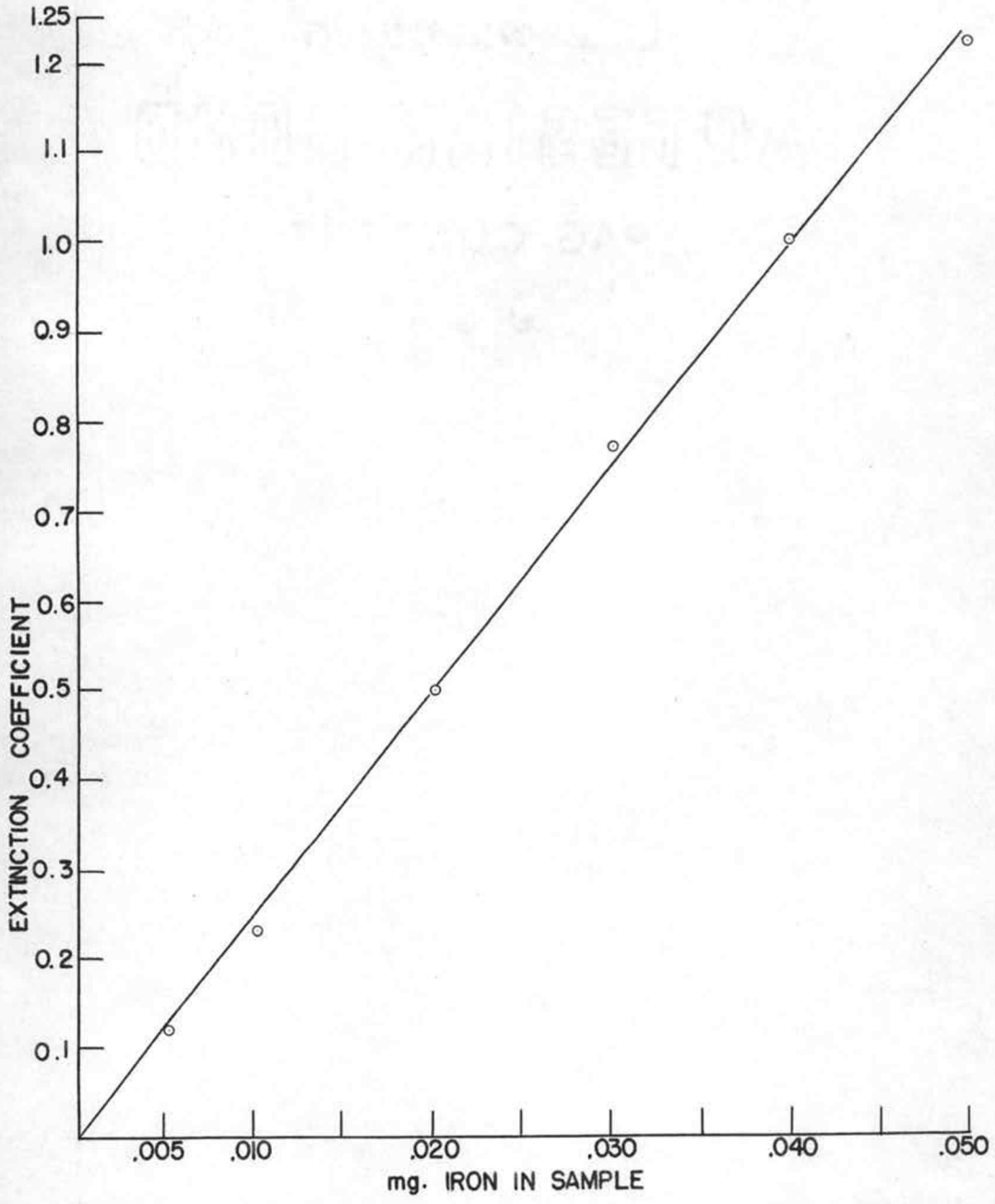
25 ml. aliquots of the diluted digestion mixture are transferred to 125 ml. separatory funnels, and 5 ml. conc. HCl, and 1 ml. 2% potassium persulphate are added to each and swirled to ensure thorough mixing. Exactly 10 ml. of 20% KSCN reagent are added to each sample, followed by exactly 25 ml. of isoamyl alcohol. The mixture is shaken for two minutes, and the color separates sharply and completely into the alcohol layer. The aqueous layer is drawn off, and 0.1 gm. anhydrous Na_2SO_4 is added to each funnel, after which the funnels are again shaken. The alcohol layer for reading is best removed from the funnel by pipette, to avoid interference by water which may remain in the spout of the funnel. The colored solutions are transferred to cuvettes and read in a Coleman spectrophotometer at a wavelength of 490mu. (see Figure 8) The results are calculated from the extinction coefficients in comparison to the standard run simultaneously. In calculation of the iron content the

results as read must be multiplied by four, as 25 ml. aliquots were taken from a total sample dilution of 100 ml.

2. Determination of Non-Hemin Iron in Liver

The method adopted for non-hemin iron in liver tissue was essentially that of Bruckmann and Zondek (20). 1 gm. of liver tissue is ground, using a mortar and pestle previously rinsed with hot, dilute HCl and distilled water. During the grinding process 5 ml. $Na_4P_2O_7$ and 10 ml. CCl_3COOH are added. When the tissue is finely divided the mixture is quantitatively transferred to a wide mouthed centrifuge tube, using a little distilled water for washing. The tube is then heated in a boiling water bath for exactly 7 minutes, removed, and centrifuged. The supernatant is decanted into a 100 ml. beaker, and the residue is washed with 4 ml. of an equal mixture of the two reagents. Washings are added to the decanted liquid. The ensuing mixture is brought to dryness on a hot plate with 10 ml. of 60% $HClO_4$ in order to convert the pyrophosphate (which interferes with thiocyanate color development) to orthophosphate. The dry white residue is dissolved in distilled water and made up to 100 ml. 25 ml. aliquots are taken for iron determination as previously outlined.

FIGURE 7 : A STANDARD CURVE FOR IRON



COLOUR DEVELOPED WITH SODIUM THIOCYANATE, EXTRACTED WITH ISOAMYL ALCOHOL

FIGURE 8 : ABSORPTION CURVE: $Fe(SCN)_3$ IN ISOAMYL ALCOHOL

PREPARED WITH STD. IRON SOL'N. CONTAINING 30 γ Fe AS FERRIC AMMONIUM SULPHATE. COLOUR DEVELOPED WITH NaSCN AND EXTRACTED IN ISOAMYL ALCOHOL. CELL 13 x 13 mm.