1953

Prophylaxis in erythroblastosis fetalis : the use of methionine

Wilmar Martin Kamprath
University of Nebraska Medical Center

PROPHYLAXIS IN ERYTHROBLASTOSIS FETALIS: THE USE OF METHIONINE

Wilmar Martin Kamprath

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

February 27, 1953

Omaha, Nebraska

TABLE OF CONTENTS

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. L. S. McGoogan for making available the clinical material presented in this manuscript.

FUNDAMENTAL PRINCIPLES OF Rh

The Rh factor received its name from the investigations carried out by Landsteiner and Wiener (1.2), who produced antisera by injecting blood of Rhesus monkeys into rabbits and guinea pigs. These antisera, after suitable absorption, were found to agglutinate the bloods of 85 per cent of the white population. By using these antisera it was possible to divide human beings into two groups. Those individuals whose red cells were clumped by the sera were said to be Rh-positive and those whose cells failed to clump were said to be Rh-negative. The original animal antisera are no longer used for Rh testing because antisera of human origin having the same specificity are now available. Shortly thereafter other antisera, also of human origin, were found. These antisera detect blood factors closely related, both serologically and genetically, to the original rhesus factor. These blood factors, together with original Rh factor, are now collectively known as Rh-Hr blood factors. Eight Rh types can be distinguished by usimg the three principal anti-Rh sera, and these can be further subdivided by the use of the two known anti-Hr sera (Wiener, 3). The factor Rh_0, whether it occurs alone or in combination, is the most antigenic, and accounts for the majority of instances of isosensitization. However, in rare susceptible individuals any of the component Rh-Hr factors may give rise to sensitization.

Genetically, each of the Rh-Hr factors behaves as a simple Mendelian dominant. With regard to Rh_0 taken alone, Rh_0-positive individuals are of two types, namely, those who are genetically "pure"

(homozygous), and those who are genetically "impure" (heterozygous). A homozygous Rh-positive husband and an Rh-negative wife will produce children, all of whom are Rh-positive (and heterozygous), while a heterozygous Rh-positive man and an Rh-negative woman will produce children, half of whom are Rh-negative like the mother, and the other half of whom are heterozygous Rh-positive like the father.

With the aid of anti-Hr sera the distinction between hetero-and homozygosity can be made with a high degree of probability, and this is of considerable importance in its clinical application. A severely sensitized Rh-negative woman with a heterozygous husband has a 50 per cent chance of having an Rh-negative child who would not be harmed by the maternal antibodies, while with a homozygous husband there is no chance at all (Wiener and Wexler, 4).

SENSITIZATION TO THE Rh FACTOR

Isoimmunization by the Rh factor occurs in two groups of cases, namely, (1) Rh-negative individuals after transfusions of Rh-positive blood, and (2) Rh-negative women immunized by Rh-positive fetal blood. The deliberate or accidental injection of Rh-positive blood into an Rh-negative individual will sensitize as many as 80 per cent of such persons depending upon the quantity of blood injected and the timing of the injection (Wiener, 5). It is now generally accepted that 92 per cent of all cases of erythroblastosis fetalis result from immunization of the Rh-negative mother by Rh-positive fetal blood and subsequent action of the maternal anti-Rh antibodies (agglutinins and blocking antibodies) on the Rh-positive fetal blood (Levine and Waller,6).

According to Levine (7), Rh-isosensitization by pregnancy occurs as the result of the passage of minute quantities of the infant's Rh-positive blood into the maternal circulation. This is most apt to occur during parturition, though it may occur at any time during pregnancy. It has been calculated by Levine (6) that the passage of as little as 0.13 cc. of fetal blood is required to immunize the Rh-negative mother.

Although immune antibodies (anti-Rh agglutinins or blocking antibodies), in contrast to normal iso-antibodies, have a comparatively short duration in the blood plasma - i.e., from several weeks to one or more years after pregnancy - the R. E. cells responsible for antibody production probably retain the sensitized state for a long time, if not permanently (Levine, 8).

INCIDENCE OF ERYTHROBLASTOSIS FETALIS

Erythroblastosis fetalis is not a leading cause of fetal or neonatal morbidity or mortality. Its incidence is directly proportional to the frequency of Rh-negative individuals in any population; in a population with 15 per cent Rh-negative individuals it will occur about once in every 150 to 200 full term pregnancies. About one fetal or neonatal death per 400 deliveries is caused by erythroblastosis fetalis (Levine, 9). Potter and Adair (10) report the incidence of erythroblastosis among the children of Rh-negative mothers at Chicago Lying-In Hospital as being 1:37, with the incidence of the fatal form being 1:56. Of 221 stillbirths occurring at the same hospital, 8.1 per cent were

due to erythroblastosis fetalis. Allen et al. (11)states that hemolytic disease, due to maternal iso-immunization by the Rh factor occurs in about 0.66 per cent of newborn infants.

CORRELATION BETWEEN ANTIBODY TITER AND THE SEVERITY OF THE DISEASE

Since the cause of erythroblastosis is the interaction of the infant's Rh-positive cells and Rh antibodies, it is to be expected that a correlation should exist between the concentration of antibodies in the maternal serum and the severity of the disease in the infant. Unfortunately, the severity of the disease in the infant is difficult to assess, and examples of severe disease with low antibody titers have been published in the literature. In addition, titrations done in different laboratories appear to vary widely.

Primrose et al. (12) believe that both the degree and duration of sensitization can be plotted graphically in such a manner as to make reasonably accurate predictions. Sacks and his associates (13) have been able to show that the stillbirth rate from erythroblastosis fetalis increases directly with the level of maternal antibody titer prior to delivery. King and King (14) state that women showing blocking antibodies early in pregnancy are very likely to deliver affected children, especially if the titer rises in the late months. Holmstrom (15) insists that the level of the maternal antibody titer is a poor index of how severely the infant will be involved. Zelenik et al. (16) found that there was no correlation between the severity of the disease in the newborn and the saline antibodies, a fairly close correlation with

the albumin antibodies, and a much more direct correlation with the anti-human globulin serum antibodies if the titer was 1:4 or more.

Page et al.(17) believe that so long as the antibodies exist to any significant degree, there is only a moderate relationship between the actual quantity present and the fetal outcome - a rapid rise or fall in the titer during the last month is not necessarily of significance.

It has been pointed out by Page, Hunt and Lucia (18) that the length of time the antibodies existed before birth was of prognostic importance, and that the critical period of time was about ten weeks. This has been confirmed by Murray and Taylor (19) who found that in twenty-two cases where antibodies existed in the first half of pregnancy, all babies were affected and only five survived; whereas in eleven cases in which antibodies were not present at sixteen weeks, but appeared after the thirtieth week, all babies survived.

Wiener and Wexler (4) believe that no absolute statement can be made regarding the prognosis based on the antenatal maternal Rh antibody titer alone in specific instances. But in general it may be stated that the more severely affected infants are usually born to mothers whose sera show the highest antibody titers, and that with low antibody titers the fetal morbidity and mortality tend to be lower. In a series of cases reported by Wiener and Wexler (4) the total mortality rate was 12.2 percent when the maternal titer was four units or less by the albumin-plasma method, while the total mortality rate was as high as 72.2 percent with titers up to 256 units.

Potter (20) believes that in her hands the previous maternal

history has been of more value in prognosticating the fate of an infant born to an immunized Rh-negative woman than have changes in maternal antibody titer or differences in the variety of antibodies present. If the antibody titer is zero, with a negative past history, the outlook is almost 100 per cent good; and prognosis is most dependably established in these cases on the basis of such a history plus a negative titer. If the titer is positive, less definite conclusions can be drawn and in that case, if a woman has had a previous erythroblastotic child, and if her husband is homozygous, the outlook is grave.

REVIEW OF PROPHYLAXIS IN ERYTHROBLASTOSIS

With maternal immunization established as the cause of hemolytic disease of the newborn, it is not unreasonable to believe that some means might be found by which the disease could be prevented. Two main lines of approach are possible: (1) the prevention of immunization of the mother, and (2) the prevention of fetal injury after maternal immunization has been established (Potter, 21).

It has been shown that immunization is ordinarily initiated at some time before the beginning of the pregnancy during the course of which the fetus is injured. Consequently, attention must be directed toward the elimination of the situations which may be conducive to the production of immunization.

No Rh-negative female should ever be transfused with Rh-positive blood. All those found to be Rh-negative must receive Rh-negative blood. One or more transfusions of Rh-positive blood may prevent a

woman, many years later, from having one or more normal Rh-positive infants. The simple procedure of checking Rh before transfusion and using Rh compatible blood should reduce the incidence of erythroblastosis fetalis, especially in its most severe forms. Levine (22) states that the incidence of erythroblastosis fetalis is almost twice as common in Rh-negative women previously immunized by transfusions, with a high incidence of fetal death in the first born of the transfused group.

The conduct of labor in Rh-negative women should be such that as little opportunity as possible is afforded for immunization. Cesarean section may permit entrance of fetal cells into the maternal circulation if the placenta is on the anterior wall and is incised when the uterus is opened. On the other hand, a long labor increases the possibility of injury to placental villi which may allow fetal blood to escape. Manual removal of the placenta also increases the liklihood of escape of fetal cells into the maternal circulation (Potter, 21).

If immunization has once been produced, there is no known way of preventing the entrance of antibodies into the fetal circulation or of preventing the union of the antibodies with the Rh-positive fetal cells. Various procedures have been recommended, but none have proven effective.

Burnham (23), believing that a lack of vitamin C might be responsible for a decrease in capillary integrity and a consequent leakage of cells from the vessels in the villi, suggested that giving large amounts of this vitamin to women who have delivered erythroblastotic infants would lower the incidence in subsequent pregnancies. If

vitamin C were to be of value, however, it would have to be given to all pregnant Rh-negative women. After the disease has occurred there is little reason to expect that vitamin C could prevent its repetition. Potter (21), used vitamin C in two cases in which it failed to prevent hemolytic disease.

In 1946 Wiener and Sonn (24) gave injections of typhoid and pertussis vaccine during pregnancy in the hope that the administration of a strong antigen might interfere with the development of antibodies in response to the weaker (Rh) antigen. They found, however, that such a procedure was of no value after immunization had once been established, a fact which would completely invalidate its therapeutic usefulness. Moloney (25) stated that the use of pertussis vaccine during pregnancy was not only useless but may be harmful.

In 1947 Kariker (26), acting upon reports that ethylene disulfonate was of value in allergic conditions, administered this chemical in minute amounts to pregnant women who had previously given birth to erythroblastotic infants. His results in three patients suggested that such treatment might have some effect in lowering the antibody titer in maternal serum. However, the dosage of ethylene disulfonate used by Kariher was so low (two trillionths of a milligram) that he himself suggested that any effect may have been due to distilled water rather than to the chemical agent. Moloney (25), in a series of twelve cases could determine no benefit from the use of ethylene disulfonate. Barglow (27) in a report of one case, expressed doubt that the lowered antibody titer in that one case could definitely be attributed to

ethylene disulfonate.

The possibility that a specific Rh hapten derived from Rh-positive red blood cells might be effective in neutralizing Rh antibodies was first suggested by Belkin and Wiener (28) in 1944. In 1947 and 1949 Carter (29, 30) claimed to have isolated from human Rh-positive red blood cells a fraction containing an Rh hapten. She reported that injections of this fraction into Rh sensitized pregnant women resulted in a fall in circulating antibody titer. Goldsmith (31) in 1950 reported three cases in which he had used Rh hapten. He described beneficial results in two of the cases. Most other workers have been unable to reproduce the findings of Carter, and their conclusion is that Rh hapten is not of clinical value in altering the fetal prognosis. Unger (32), Wolf et al. (33), Greenwalt (34), Hamilton and Brockland (35), Spurling et al. (36), and Marsters, Schmidt and Black (37) all reported unsuccessful efforts to inhibit antibody formation by the use of Rh hapten. Levine (9) in 1952 stated that "there is no evidence that any true hapten has been isolated from human Rh-positive blood, nor has there been any confirmation of reports on the value of a presumed hapten in the prevention of hemolytic disease."

The artificial insemination of an immunized woman with sperm cells from an Rh-negative man is one method of obtaining a child free from erythroblastosis. All Rh-negative children will be unaffected regardless of the degree of maternal immunization (Diamond, 38). Potter and Willson (39) have reported the birth of normal children

to women who have been artificially inseminated after having given birth to infants with hemolytic disease previously.

Homburger (40) in 1946 reported a study on guinea pigs and rabbits in which the formation of anti-Rh agglutinins following the injection of rhesus monkey blood cells appeared to be diminished when sodium salicylate had been administered three days prior to and during the period of immunization. McLennan et al. (41) reported on the use of salicylates in one case which resulted in a macerated stillborn fetus.

Hoffman and Edwards (42) in 1950 reported a series of cases in which they had used Pranone (Schering), 10 mgm. per day orally, and vitamin K, 4 to 8 mgm. I.M. weekly, in an attempt to prevent erythroblastosis fetalis. In the three patients in the series who had antibody titers before treatment, the titers fell during treatment. In the patients who had no demonstrable titers, no titers developed during treatment. Three untreated patients developed antibodies during pregnancy. The fact that all the patients who started their pregnancies without antibodies failed to develop antibodies while taking Pranone is dubious evidence of its effectiveness, for it is known that, without any treatment, only about five per cent of Rh-negative women will become sensitized, even though they are mated to Rh-positive men and are subjected to repeated pregnancies (Holmstrom, 15). Holmstrom, in an attempt to confirm the observations of Hoffman and Edwards, administered Pranone to a group of 35 pregnant, Rh-sensitized patients. The dosage averaged 100 mgm. daily for 12 weeks.

He discovered no evidence to indicate that Pranone will influence the outcome of a pregnancy complicated by Rh sensitization.

The underlying rationale of administering ACTH and/or cortisone to Rh-negative pregnant women is explained on the following grounds by Anderson, Barr and Slessor (43). These men point out that among the numerous trials of ACTH and cortisone in human disease, those which seem most relevant to the present question concern the condition of idiopathic acquired hemolytic anemia in which the hemolytic process is associated with a positive direct Coombs reaction. Here, it seems reasonable to assume that the patient develops auto-antibody, which, attaching itself to the red blood cells, is responsible for the increased rate of their destruction. There have been many reports of favorable effects of ACTH and cortisone in these cases. There is thus evidence that ACTH may have a beneficial effect in cases of hemolytic anemia in which there is auto-antibody attached to the red blood cells. In the single case history reported by Anderson, Barr and Slessor (43), in which 100 mgm. of cortisone was given daily from the thirty-third to the thirty-seventh weeks, the baby had to have an exchange transfusion despite the prenatal cortisone. In nine out of the ten surviving infants of treated sensitized mothers reported by Hunter and Ross (44), transfusions were also necessary. They gave 100 mgm of cortisone daily from the time the mother entered the hospital for delivery. Christensen, Margulis and Stewart (45) used dosages of 75 to 100 mgm. of either ACTH or cortisone given three to six times each week for periods varying from three days to six months. Two of the eight Rh-positive

babies in this series succumbed to erythroblastosis although no transfusions were deemed necessary. Holmstrom (15), who reported a series of four cases in which 100 mgm. ACTH was given daily for seven to ten days, found no change in antibody titer and all four patients ultimately delivered stillborn erythroblastotic infants. Harkins (46) reported one case in which he felt he had obtained beneficial results from the use of ACTH in 100 mgm. daily doses for three weeks prior to delivery. ACTH and cortisone may prove to be of some benefit in the prevention of erythroblastosis, but at this time, as Christensen et al. (45) have pointed out, "no significant clinical interpretation can be drawn from so few cases."

Premature delivery has been widely used as a prophylactic measure during the past few years. By reducing the duration of time that the maternal antibodies have to act on the fetal red blood cells, it is felt that the infant, even though premature, has a better chance for survival. Wiener and Wexler (4) state that a premature erythroblastotic infant who can be given the advantage of exchange transfusion has a better chance of survival than a more mature baby in whom the disease has progressed and who, as a result, might die in utero before term. They feel that Cesarean section should not be resorted to unless there is a maternal indication or a fetal indication other than erythroblastosis. Potter (21) concurs in this viewpoint for the reason stated previously, i.e., Cesarean section may permit entrance of fetal cells into the maternal circulation if the placenta is on the anterior wall and is incised when the uterus is opened. Wiener and Wexler (4) feel that a history of the birth of a previous erythroblastotic infant who

did not survive is a more important indication for early delivery than is the height of the univalent antibody titer. On the other hand, Vogel and Rosenfield (47) suggest induction between the thirty-seventh and thirty-eighth weeks if there is a significant rise in titer late in pregnancy. Philpott et al. (48) considers interuption of pregnancy in the thirty-sixth week if the case history denotes repeated fetal mishaps, and if the husband is not established as heterozygous.

PRINCIPLE OF THE USE OF METHIONINE IN ERYTHROBLASTOSIS FETALIS

Erythroblastosis fetalis occurs in one of three forms: (1) Congenital hydrops in which effusions occur in the serous cavities, with severe edema of the organs; (2) Icterus gravis, in which jaundice, occurring within the first day or two, dominates the clinical picture; and (3) anemia of the newborn, in which there is severe anemia with erythroblastosis. In addition to the edema and serous effusions the main lesions are extramedullary hematopoiesis and damage to the liver (Richter, 49). Potter (21) states that the liver ordinarily appears to be more profoundly and constantly affected than any other organ in the body, except the spleen. In the liver there are often numerous foci of developing blood cells associated with degenerative changes in the liver cells, and diffuse fibrosis. The liver cells occasionally show a mild degree of fatty metamorphosis and infrequently large amounts of fat may be found. The increase in intralobular connective tissue, which occasionally appears, was described by Henderson (50) and Harrison and Meacock (51). Hawksley and Lightwood

(52) found an increase in intralobular connective tissue in three of five infants who survived more than five weeks. The damage to the liver, when once sustained, is thought to be very long lasting, as shown by the follow-up on the cases of Philpott et al. (53) and by the work of Drummond and Watkins (54) in England. The latter two showed that the liver damage in young persons who were thought to be suffering from idiopathic congenital cirrhosis of the liver was in fact due to the late effects of erythroblastosis due to Rh incompatibility.

Taking these facts into consideration, it seemed reasonable to Philpott and his associates (53) to "attempt the protection of such fetal livers by the use of methionine whose action as a liver protector had been the subject of so much recent investigational work." Normal liver and kidney functions can be maintained only when there is adequate protein intake (Miller, 55), and as has been shown by Sims (56), there is a significantly higher excretion of methionine in pregnant women than in non pregnant women. The excretion of methionine falls off somewhat in the last trimester and this Sims attributes to some degree of methionine depletion of the maternal organism at this time.

Although, while increasing the methionine level in the mother and the fetus, the antibody development occurs as usual in the mother, and hemolysis of the red blood cells occurs in the fetus, the resulting anemia can be treated. But a severely damaged liver cannot adequately be treated.

REPORTS OF THE USE OF METHIONINE IN THE LITERATURE

Reports in the literature concerning the use of methionine as a prophylactic measure in erythroblastosis fetalis are not numerous. A total of only nineteen cases have been described. Sixteen of these are contained in the original report by Philpott et al. (53), in which the principle on which he based his treatment is explained. King and King (14) used methionine, as suggested by Philpott, in three pregnant women. One of these delivered an Rh-positive baby so severely affected by erythroblastosis that it did not survive, and the other two were Rh-negative.

Philpott used a daily dosage of five grams of crystalline methionine, and this was begun as soon as the first signs of immunization to the Rh factor were detected. He reasoned that such a dosage would provide a therapeutic systemic concentration to deal with modcrate liver damage. Any methionine obtained in foodstuffs was regarded merely as a supplement.

It was assumed that in the normal course of fetal development, as the need for methionine was manifested in the fetus, then the positive balance available in the maternal organism would be drawn across the placenta of the fetus. This is in accord with the generally accepted and proved theory of fetal demand and maternal supply. Since methionine is an essential amino acid, its passage across the placenta would be uninhibited.

Excepting three patients who had been severely immunized by recent blood transfusions, all others had had experience of previous erythroblastotic infants. On the basis of duration and height of maternal antibody titers the patients were divided into five groups (Primrose et al., 12). In each group were methionine treated patients and controls. In the three groups in which the prognosis was most serious, as shown by the controls, the difference could be seen. In these three groups, nineteen control cases had one survivor equaling 5.25 per cent survival. Of twelve cases receiving methionine, ten babies were born alive, and eight survived, giving a gross survival rate of 66.6 per cent.

While it is true that no significant clinical interpretation can be drawn from so few cases, the results appear to warrant further trial of this method of treatment.

REPORT ON TWELVE PREGNANCIES IN WHICH METHIONINE WAS USED

METHODS

The cases reported in this paper are from the private practise of Dr. L. S. McGoogan and represent all patients treated with methionine between 1947 and 1952. The methionine used was furnished through the courtesy of the Eli Lilly Company.

In evaluating any method of prophylactic treatment in erythroblastosis fetalis, it is considered essential by most authors to include only those patients who have had previous erythroblastotic infants (Moloney, 25, Anderson et al.,43, Marsters et al., 37), and who show a maternal antibody titer (Wiener, 4). Potter (20) feels that a history of previous erythroblastotic infants is of greatest importance.

All patients in this series had experience of previous erythroblastotic babies, or babies bron dead as a result of the disease. Ten of the pregnancies had been preceded by erythroblastosis which resulted in death of the infant. Four of these were stillborn.

Of the ten husbands in the group, seven were presumably Rh-positive homozygous while three were heterozygous. These determinations, as well as most of the antibody titrations, were done in the laboratory of Dr. Philip Levine at the Ortho Laboratories in Raritan, N. J. In the first pregnancy of Case 3 and in Case 4 the antibody determinations were made in the Nebraska State Laboratories in Lincoln.

The antibody titers were determined at the initial visit to the office, and after the twenty-eighth to thirtieth weeks the titrations were done at approximately two to four week intervals.

Methionine was given in daily amounts varying between 1.5 and six grams and for periods of seven to 28 weeks. Total dosages varied between 147 grams and a maximum of 840 grams. No case of idiosyncrasy to the drug was noted. Constant check was made on the mothers' consumption of the drug.

Samples of cord blood from the liveborn group were examined, and the presence of hemolytic disease of the newborn was confirmed by the pediatrician. The deadborn group was subjected to careful pathologic examination and the nature of their disease studied.

The grading system used by Primrose, Van Dorsser and Philpott (12) is included in the table for better comparison with the results of the series reported by Philpott et al. (53).

DATA ON CASES TREATED WITH METHIONINE

CASE NO.	NAME	HOSPITAL RECORD	AGE	PARA	GRAV	HUSBAND'S PROBABLE Rh GENOTYPE	PREVIOUS ERYTHROBLASTOSIS STILLBORN	PREVIOUS ERYTHROBLASTOSIS SURVIVAL WITH TREATMENT	PREVIOUS ERYTHROBLASTOSIS DIED POST PARTUM	GROUP (PHILPOTT)	METHIONINE AMT DAILY	METHIONINE DURATION	BLOCKING ANTIBODIES ALB. AGGLUTININS WEEK	BLOCKING ANTIBODIES ALB. AGGLUTININS TITER	PREVIOUS ERYTHROBLASTOSIS STILLBORN	PREVIOUS ERYTHROBLASTOSIS SURVIVAL WITH TREATMENT	PREVIOUS ERYTHROBLASTOSIS DIED POST PARTUM	DELIVERY	AUTOPSY	CEPH-CHOL FLOCC.	COOMB'S TEST	HEPATO-MEGALY	PREVIOUS TRANSFUSIONS
1	M.N.	Imm. 1214-51	23	II	IV	Positive Homozygous	1			V	3 Gm.	16 Wks	12 28 34	1:256 1:64 1:32		Exchange Transfusion		Cesarean Term		24 hrs + 48 hrs -	Positive	0	1944 Rh Type Unknown
1	M.N.	Imm. 1024-52	24	III	V	Positive Homozygous	1			IV	3 Gm.	24 Wks	28 32 36	1:64 1:128 1:128		Exchange Transfusion		Cesarean 37th Wk		24 hrs - 48 hrs -	Positive	+++	1944 Rh Type Unknown
2	H.M.	Imm. 2431-51	27	I	IV	Positive Homozygous	2			IV	3 Gm.	29 Wks	7 30 33 35	1:4 1:512 1:512 1:512		Two Small Transfusions		Cesarean 37th Wk			Positive	0	
3	K.D.	St. Jos. 12/5/47	30	II	III	Positive Homozygous			1	II	5 Gm.	11 Wks	29 32 33 34	Neg. 1:1 1:1 1:8		13 Small Transfusions		Cesarean 37th Wk					
3	K.D.	Imm. 1153-51	33	III	IV	Positive Homozygous			1	V	5 Gm.	25 Wks	12 26 32 34	1:512 1:64 1:32 1:32		Exchange Transfusion		Cesarean 37th Wk		24 hrs - 48 hrs -	Positive	0	
4	E.O.	Meth. 8/10/48	31	II	IV	Positive Heterozygous	1			II	3 Gm.	7 Wks	29 32 34	Neg. Neg. 1:2			During Transfusion	Cesarean 37th Wk	E.F.				
5	R.S.	Imm. 2939-52	27	II	III	Positive Heterozygous			1	III	2 Gm. 6 Gm.	4 Wks 9 Wks	Prior to Preg. 18	1:512 1:32	31 Wks			Spont.	Extreme Maceration				
6	G.B.	Imm. 3785-51	29	II	III	Positive Homozygous			1	V	1:5 Gm.	30 Wks	10 24 34	1:16 1:256 1:256	38 Wks			Spont.	Extreme Maceration				
7	B.S.	Imm. 3388-49	34	V	V	Positive Homozygous			3	IV	5 Gm.	16 Wks	20 26 29 33 35	1:64 1:16 1:64 1:32 1:128			During Transfusion	Induction 36th Wk	E.F.	24 hrs - 48 hrs -	Positive	+	
8	C.C.	Imm. 5468-52	26	II	III	Positive Homozygous		1		II	3 Gm. 5 Gm.	15 Wks 15 Wks	15 26 30 38	Weak Anti-D Neg. 1:2 1:8		No Transfusion Needed		Spont. 39th Wk			Positive	0	
9	K.O.	Imm. 5742-49	29	III	IV	Positive Homozygous		2		IV	3 Gm. 4 Gm. 5 Gm.	4 Wks 4 Wks 4 Wks	27 31	Neg. 1:128	31 Wks			Spont.	E. F.			+	
10	P.M.	Imm. 2320-52	30	III	VII	Positive Heterozygous			1	III	2 Gm.	19 Wks	30	1:32			30 Min. After Birth	Cesarean 30th Wk *	E.F. Fetal Hydrops		Positive	+++	I.M. Blood as a child

*Bleeding central placenta praevia

CASE HISTORIES

Case 1 - Mrs. M. N. had two normal Rh-negative girls in 1944 and 1946 by her first husband. During her first labor, in 1944, she was given a blood transfusion of unknown Rh type. In 1950, after marrying an Rh-positive homozygous man, she delivered a stillborn erythroblastotic child. In 1950, she was seen in the office in the twelfth week of her fourth pregnancy with a blocking antibody titer of 1:256. At twenty-four weeks she was started on three grams of methionine daily which was continued to term. In the twenty-eighth week the antibody titer was 1:64 and in the thirty-fourth week it was 1:32. She was delivered by cesarean section at term of an Rh-positive erythroblastotic girl who was given an exchange transfusion and survived. The cephalin-cholesterol flocculation was one plus at twenty-four hours and negative at forty-eight hours with no enlargement of liver or spleen.

In 1951 the patient was seen in the thirteenth week of her fifth pregnancy when she was again started on three grams of methionine daily. Her blocking antibody titer in the twenty-eighth, thirty-second and thirty-sixth weeks were 1:64, 1:128, and 1:128 respectively. She was delivered by cesarean section in the thirty-seventh week. The Rh-positive erythroblastotic infant was given an exchange transfusion and survived. The cephalin-cholesterol flocculation was negative in 24 hours with a trace at 48 hours. The liver and spleen were markedly enlarged.

Case 2 - Mrs. H. M. had a normal Rh-positive child in 1944, an erythroblastotic stillborn child in January of 1946 and an erythroblastotic stillborn in December of 1946. Her husband was Rh-positive homozygous. In 1950, in the seventh week of her fourth pregnancy, her blocking antibody titer was 1:4. In the thirtieth, thirty-third and thirty-fifth weeks the blocking antibody titer was 1:512. In the eleventh week of pregnancy she was started on three grams of methionine daily. She was delivered by cesarean section in the thirty-seventh week. The Rh-positive erythroblastotic baby was given two small transfusions and survived. The liver and spleen were not abnormal in size.

Case 3 - Mrs. K. D. had a normal Rh-positive child in 1944, and an Rh-positive erythroblastotic child who died four days after birth in 1946. Her husband was Rh-positive homozygous. In 1947 she was seen in the eleventh week of her third pregnancy with no Rh antibody titer. She was started on five grams of methionine per day. The blocking antibody titers in the twenty-ninth, thirty-second, thirty-third and thirty-fourth weeks were negative, 1:1, 1:1 and 1:8 respectively. She was delivered by cesarean section in the thirty-seventh week. The infant was Rh-positive and erythroblastotic, but survived with thirteen small transfusions.

The patient was seen again in 1950 in the twelfth week of her fourth pregnancy. Her blocking antibody titer was 1:512 at that time. She was started on five grams of methionine daily in the twelfth week. The blocking antibody titers in the twenty-sixth,

thirty-second and thirty-fourth weeks were 1:64, 1:32 and 1:32 respectively. She was delivered by cesarean section in the thirty-seventh week. The infant was Rh-positive and erythroblastotic but survived with exchange transfusion. The cephalin-cholesterol flocculation was negative at 24 and 48 hours.

Case 4 - Mrs. E. O. had a normal Rh-positive boy in 1938 and an abortion at three months in 1938. In 1939 she was delivered of a stillborn erythroblastotic infant with marked hepatosplenomegaly. Her husband was Rh-positive heterozygous. In 1948 she was seen in the twenty-ninth week of her fourth pregnancy with a negative Rh antibody titer. She was started on three grams of methionine daily in the twenty-ninth week. The blocking antibody titer was again negative in the thirty-second week. In the thirty-fourth week the blocking antibody titer was 1:2. She was delivered by cesarean section in the thirty-seventh week. The condition of the Rh-positive infant was good at birth, but jaundice developed rapidly and she died before a transfusion could be started. Autopsy showed typical erythroblastosis fetalis.

Case 5 - Mrs. R. S. had a normal Rh-positive child in 1948 and an Rh-positive erythroblastotic infant in 1950 who died during exchange transfusion, shortly after birth. Her husband was Rh-positive heterozygous. In 1951 her blocking antibody titers varied between 1:1000 and 1:512. When seen in the eighteenth week of her third pregnancy in 1952 the blocking antibody titer was 1:32. She was started on two grams of methionine in the eighteenth week and

this dosage was increased to six grams in the twenty-second week. In the thirty-first week of pregnancy she delivered spontaneously a stillborn macerated fetus which appeared grossly to be erythroblastotic. Extreme maceration prevented a definite microscopic diagnosis of erythroblastosis fetalis.

Case 6 - Mrs. G. B. had a normal Rh-positive girl in 1948, and an erythroblastotic infant in 1949 who died 48 hours after birth in spite of transfusion. Her husband was Rh-positive homozygous. She was seen in 1951 in the tenth week of her third pregnancy with a blocking antibody titer of 1:16. She was started on a daily dose of 1.5 grams of methionine. In the twenty-fourth and thirty-fourth weeks her antibody titer was 1:256. She went into spontaneous labor in the thirty-eighth week and delivered a macerated stillborn infant. The placenta showed marked degenerative changes similar to those seen in erythroblastosis fetalis. Definite microscopic diagnosis was not possible at autopsy due to extreme maceration.

Case 7 - Mrs. B. S. had two normal Rh-positive boys in 1938 and 1941. In 1944 she had an Rh-positive erythroblastotic boy who lived 24 hours. In 1946 she had Rh-positive erythroblastotic twin boys who lived only four hours in spite of transfusion. Her husband was Rh-positive homozygous. In 1949 she was seen in the twentieth week of her fifth pregnancy with a blocking antibody titer of 1:64. Her blocking antibody titers in the twenty-sixth, twenty-ninth, thirty-third and thirty-fifth weeks were 1:16, 1:64, 1:32 and 1:128 respectively. She was started on five grams of methionine daily in the

twentieth week. Labor was induced in the thirty-sixth week, and an Rh-positive erythroblastotic girl was delivered. An exchange transfusion was started, but the infant became cyanotic and died. The cephalin-cholesterol flocculation was negative at 24 and 48 hours. The liver and spleen were both enlarged and a diagnosis of erythroblastosis fetalis was established at autopsy.

Case 8 - Mrs. C. C. had a normal Rh-positive child in 1942. In 1947 she had an Rh-positive erythroblastotic infant who survived with transfusions. Her husband was Rh-positive homozygous. She was started on three grams of methionine daily in the ninth week of her third pregnancy. In the fifteenth week she had a weak antibody titer and in the twenty-sixth week the blocking antibody titer was negative. In the thirtieth and thirty-eighth weeks the blocking antibody titers were 1:2 and 1:8 respectively. The daily methionine dosage was increased to five grams in the twenty-fourth week. She delivered spontaneously in the thirty-ninth week. The Rh-positive infant showed no signs of erythroblastosis and did well without transfusion.

Case 9 - Mrs. K. O. had a normal Rh-positive girl in 1939 and Rh-positive erythroblastotic children in 1944 and 1946. Both of the latter survived with small transfusions. Her husband was Rh-positive homozygous. In 1950 she was seen in the twenty-seventh week of her fourth pregnancy with a negative Rh antibody titer. In the thirty-first week the blocking antibody titer was 1:128. She was put on three grams of methionine daily in the nineteenth week, four grams daily in the twenty-third week and five grams daily in the twenty-seventh week.

She went into labor in the thirty-first week and delivered a stillborn, erythroblastotic infant with marked edema. The liver and spleen were both enlarged and autopsy established the diagnosis of erythroblastosis fetalis.

Case 10 - Mrs. P. M. had a history of I. M. injections of blood of unknown Rh type as a child. She had an abortion in 1943, an Rh-negative normal child in 1944 and an Rh-positive erythroblastotic infant in 1945 who died three days after birth. In 1946 she delivered a stillborn infant in the sixth month. In 1947 she had an Rh-positive erythroblastotic boy who survived. In 1951 she had a premature separation of the placenta at five months with another fetal death. She was seen in 1952 in the eleventh week of her seventh pregnancy and was started on two grams of methionine daily. In the thirtieth week her blocking antibody titer was 1:32. In the thirtieth week she began bleeding from a central placenta praevia. A cesarean section was done and a living, extremely edematous erythroblastotic infant was delivered. The liver and spleen were both markedly enlarged. The infant died 30 minutes after delivery. The baby weighed 2470 grams and the placenta weighed 1020 grams.

DISCUSSION

In this series of twelve pregnancies in ten patients, methionine was given in daily doses of from 1.5 to six grams over periods of seven to 28 weeks. (Three additional patients with histories of erythroblastotic children were also given methionine, but these delivered Rh-negative normal infants, and are merely mentioned in passing. It is interesting to note, however, that one of these patients had a blocking antibody titer of 1:64 in the seventh week which rose to 1:128 in the thirtieth week. Yet she delivered a normal Rh-negative child.)

Of the twelve pregnancies reported, three resulted in erythroblastotic stillborn infants. All nine of those born alive were erythroblastotic, and of these nine six survived. Two babies died during exchange transfusion, probably due to errors in technique, while the extremely hydropic infant in Case 10 died almost immediately after birth. This gives a gross survival rate of 50 per cent.

Potter (20) in 1948 reported on 179 pregnancies in 96 women which followed the birth of an infant with erythroblastosis. These resulted in three Rh-negative, 144 Rh-positive infants with erythroblastosis and 32 abortions. Of the Rh-positive infants, 69 were stillbron, 63 died and only 12 survived. This represents less than 10 per cent survival as compared with 50 per cent in this series.

Nine of the pregnancies were placed in groups III to V (Philpott et al. 12) on the basis of height and duration of antibody titer. Of these nine pregnancies, three resulted in erythroblastotic stillbirths. Of the six erythroblastotic infants born alive, four survived.

This gives a 44.4 per cent survival as compared with 66.6 per cent in Philpott's twelve cases in the same groups. It is to be noted that there was only one survivor out of nineteen in Philpott's control group in grades III to V (53).

It should be pointed out that early delivery is an important factor which must be considered in the end results when one is evaluating a method of treatment. However, in comparing the results of different series of cases in which early delivery is routine, the method of delivery becomes more important. Early delivery is quite generally employed. But it is important to note whether the early delivery is via induction or cesarean section. It has been mentioned previously that many workers feel that cesarean section is not justified unless there is a maternal or fetal indication other than erythroblastosis. Five of the six surviving infants in this series were delivered by cesarean section in the thirty-seventh week of pregnancy. It was felt that the chances of survival of the baby were better at this time than if the pregnancy were allowed to continue to term. Induction of labor in these cases was thought to be contraindicated because of lack of effacement and dilatation of the cervix. Two mothers, each of whom had experience of previous fetal erythroblastotic deaths, were delivered by cesarean section in four pregnancies, and all four babies survived.

SUMMARY

1. The fundamental principles of Rh and their development are discussed briefly. The genetic basis for homo - and heterozygosity is mentioned.

2. The two groups of cases in which isoimmunization by the Rh factor occurs are mentioned. The importance of Rh compatible transfusions of blood is stressed. By far the greatest number of cases of isoimmunization by the Rh factor occur during pregnancy.

3. The incidence of erythroblastosis fetalis is not great, but the individual sensitized mother who repeatedly produces erythroblastotic infants presents a serious and challenging problem.

4. The correlation which exists between antibody titer and the severity of the disease in the infant is at best a general one and cannot be relied on absolutely in the individual case. Greater emphasis is placed by some authors on the previous erythroblastotic history and the zygosity of the father in the matter of prognosis for the infant.

5. A brief review of the literature as regards various methods of prophylaxis in erythroblastosis is presented. The use of Rh compatible blood for transfusions is of prime importance. The idea that early delivery is beneficial is generally accepted, but the method of delivery - i.e. induction or cesarean - still is disputed by various workers. The use of drugs of various kinds has been generally unsuccessful. The use of ACTH and cortisone should probably be given further trial before final judgment is passed on

its efficacy.

6. The principle of the use of methionine as a liver protector in erythroblastosis fetalis is discussed.

7. Review of the literature concerning the use of methionine in erythroblastosis fetalis. A total of only nineteen cases have been reported.

8. Twelve cases in which methionine was used are presented. The methods of selection of patients on the basis of previous erythroblastotic history is emphasized. The amounts of methionine used, and the results, are tabulated. The individual case histories are given, with elaboration on the information contained in the table. The gross survival rate was 50% of all babies, and 66.6% survival of those born alive. The method and time of delivery are considered.

CONCLUSIONS

1. It is generally accepted that attempts to reduce the antibody titers in Rh-negative pregnant women the use of various drugs have so far been unsuccessful.

2. Rh compatible blood for transfusions is mandatory.

3. Early delivery is generally accepted as a valuable adjunct in the handling of Rh-negative pregnant women who present a history of erythroblastotic infants.

4. The supposition that cesarean section results in an increased mortality rate in erythroblastosis fetalis would appear to be fallacious on the basis of the cases presented.

5. While it is difficult to draw any significant conclusions from the few cases presented, the survival of 50 per cent of the infants who were treated with methionine and delivered early by cesarean section compares favorably with the survival of erythroblastotic infants in comparable situations where methionine was not used.

6. The results appear to warrant further trial of methionine as a prophylactic measure in erythroblastosis fetalis.

BIBLIOGRAPHY

1. Landsteiner, K., and Wiener, A. S.: An Agglutinable Factor in Human Blood Recognized Immune Sera for Rhesus Blood, Proc. Soc. Exper. Biol. and Med. 43: 223, Jan. 1940.

2. Landsteiner, K., and Wiener, A. S.: Studies on an Agglutinogen (Rh) in Human Blood Reacting with Anti-Rhesus Sera and with Human Isoantibodies, J. Exper. Med. 74: 309, Oct. 1941.

3. Wiener, A. S.: Recent Developments in the Knowledge of the Rh-Hr Blood Types; Tests for Rh Sensitization, Am. J. Clin. Path. 16: 477, Aug. 1946.

4. Wiener, A. S., and Wexler, I. B.: Management of the Isosensitized Pregnant Patient and her Erythroblastotic Infant, M. Clin. No. Am. 35: 749, May 1951.

5. Wiener, A. S.: Further Observations on Isosensitization to the Rh Factor, Proc. Soc. Exper. Biol. and Med. 70: 576, April 1949.

6. Levine, P., and Waller, R. K.: Prevention of Erythroblastosis Fetalis in First Born: Prevention of Most Severe Forms, Blood 1: 143 - 155, March 1946.

7. Levine, P., Burnham, L., Katzin, E., and Vogel, P.: The Role of Isoimmunization in the Pathogenesis of Erythroblastosis Fetalis, Am. J. Obst. and Gynec. 42: 925, Dec. 1941.

8. Levine, P.: Mechanism of Iso-immunization
Red Blood Cells: Standardization of Anti-Rh Serums, the Rh Factor of Arch. Path. 37: 83, February 1944.

9. Levine, P.: Rh Studies in the Mother in Reducing Infant Mortality, N. Y. State J. Med. 52: 4, 15 February 1952.

10. Potter, E., and Adair, F.: Fetal and Neontal Death, University of Chicago Press, 1949.

11. Allen, F. H., Diamond, L. K., and Watrous, J. B., Jr.: Erythroblastosis Fetalis; value of blood from female donors for exchange transfusions, New England J. Med. 241: 799, 1949.

12. Primrose, T., Van Dorsser, G., and Philpott, N. W.: A Graphic Method of Prognosis for the Infant in the Antenatal Case of Rh Isoimmunized Pregnant Women. Am. J. Obst. and Gynec. 54: 662, October 1947.

13. Sacks, M. S., Kuhns, W. J., and Jahn, E. F.: Studies in Rh Isoimmunization in Pregnancy, Am. J. Obst. and Gynec. 54: 400, September 1947.

14. King, E. L., and King, J. A.: Rh Factor in 1000 Consecutive White Obstetrical Patients, Am. J. Obst. and Gynec. 622: 758, October 1951.

15. Holmstrom, E. G.: Experience with Pranone and ACTH in Rh-sensitized Pregnancies, Am. J. Obst. and Gynec. 63: 5, May 1952 p. 1038.

16. Zelenik, L., Altamirano, M., Prystowsky, H.: Studies on Rh Evaluation of Anti-human Globulin Serum in Hemolytic Disease of the Newborn, Am. J. Obst. and Gynec. 64: 365, 1952.

17. Page, E., Hunt, M., Lucia, S.: Observations on Antepartum Prediction of Hemolytic Disease of the Newborn, V. of Pediatrics 37: 4, p. 656, October 1950.

18. Page, E., Hunt, M., Lucia, S.: The Antepartum Prediction of Hemolytic Disease of the Newborn, Am. J. Obst. and Gynec. 52: 794, 1946.

19. Murray, J., and Taylor, M.: Rh Immunization in Pregnancy, J. Obst. and Gynec. Brit. Emp. 56: 741, 1949.

20. Potter, E. L.: Reproductive Histories of the Mothers of 322 Infants with Erythroblastosis, Pediat., 2: 369 - 381, October 1948.

21. Potter, E.: Rh: Its Relation to Congenital Hemolytic Disease and to Intragroup Transfusion Reactions, Chicago, 1947, The Year Book Publishers, Inc.

22. Levine, P.: Prevention of Unintentional Iso-immunization of the Rh-negative Female Population, J. A. M. A. 128: 946, July 28, 1945.

23. Burnham, L.: Vitamin C Deficiency as a Possible Factor in the Pathogenesis of Erythroblastosis Fetalis, Am. J. Obst. and Gynec. 44: 920, 1942.

24. Wiener, A. and Sonn, E.: Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis): II Illustrative Case Histories of Rh Sensitization. Am. J. Dis. Child. 7: 25, January 1946.

25. Moloney, W. C.: Attempts at Desensitization of Women Immunized by the Rh Factor: Use of Ethylene Disulfonate, Am. J. Obst. and Gynec. 60: 616, September 1950.

26. Kariker, D.: Prophylaxis of Hemolytic Disease by Intramuscular Injection of Ethylene Disulfonate into Mother, Am. J. Obst. and Gynec. 54: 1, July 1947.

27. Barglow, D. R.: Ethylene Disulfonate and Hemolytic Disease of the Newborn, Rocky Mt. Med. J. 46: 938, November 1949.

28. Belkin, R. and Wiener, A.: Demonstration of the Properties A, B, M, N and Rh in Red Cell Stromata, Proc. Soc. Exper. Biol. and Med. 56: 214, 1944.

29. Carter, B. B.: Preliminary Report on a Substance which Inhibits Anti-Rh Serum, Am. J. Clin. Path. 17: 646, 1947.

30. Carter, B. B: Rh Hapten: Its Preparation, Assay, and Nature. J. Immunol. 61: 79, 1949.

31. Goldsmith, J.: Experiences with Rh hapten, Am. J. Obst. and Gynec. 59: 172, January 1950.

32. Unger, L. J.: Studies on Preventive Treatment of Rh Sensitization, Am. J. Obst. and Gynec. 58: 1186, December 1949.

33. Wolf, A. M., Schultz, C., Freundlich, M. and Levinson, S. O.: Clinical Study of Rh hapten, J. A. M. A. 144: 88, September 9, 1950.

34. Greenwalt, T. J.: Critical Appraisal of Present Status of "Rh hapten," Wisconsin Med. J. 49: 711, August 1950.

35. Hamilton, E. G., and Brockland, M. E.: Results of Treatment with Rh hapten, Am. J. Obst. and Gynec. 60: 813, October 1950.

36. Spurling, C. L., Sacks, M. S., and Jahn, E. F.: Influence of Rh hapten Therapy on Course of Rh Isosensitization in Pregnancy, Blood 5: 1125, December 1950.

37. Marsters, R., Schmidt, R., and Black, M.: Efficacy of Rh hapten Therapy in Prevention of Erythroblastosis Fetalis, Am. J. Obst. and Gynec. 63: 3, March 1952.

38. Diamond, L.: The Clinical Importance of the Rh Blood Type, New England J. Med. 232: 447, April 19 and 475, April 26, 1945.

39. Potter, E., and Willson, J.: Artificial Insemination as a Means of Preventing Erythroblastosis Fetalis, J. A. M. A. 127: 458, February 24, 1945.

40. Homburger, F.: Sodium Salicylate Inhibiting Anti-Rh Immunization in Animals, Proc. Soc. Exper. Biol. and Med. 61: 101, 1946.

41. McLennan, C., Jager, B. and Matson, G.: Salicylates in Prevention of Erythroblastosis Fetalis, Am. J. Med. 3: 661, November 1947.

42. Hoffman, P. B., and Edwards, D. E.: Preliminary Report on a New Concept of Treatment of Rh-negative Pregnant Women, Am. J. Obst. and Gynec. 59: 207, Van. 1950.

43. Anderson, J. R., Barr, G. M., and Slessor, A.: Attempt to Prevent Erythroblastosis Fetalis by the use of Cortisone during Pregnancy, Brit. Med. J. 2: 543, 1952.

44. Hunter, O. B. and Ross, J. B.: Cortisone in Rh Incompatibilities, South. Med. J. 45: 732, 1952.

45. Christensen, R. C., Margulis, R. R. and Stewart, H. L.: The Effect of ACTH and Cortisone on the Rh_0 Antibody Titer during Pregnancy. West. J. Swg. 60: 429, 1952.

46. Harkins, J. E.: ACTH in Treatment in Utero; a Case Report, Permanente Found. M. Bull. 9: 4, October 1951.

47. Vogel, P. and Rosenfield, R.: The Importance of Rh Studies in Mother and Child in Reducing Infant Mortality, N. Y. State J. Med. 52: 4, 15 February 1952.

48. Philpott, N. W., Latour, J. and Van Dorsser, G.: Review of Cases of Rh Iso-immunization during the past Five Years at the Royal Victoria Maternity Hospital, Am. J. Obst. and Gynec. 52: 926, 1946.

49. Richter, M. N.: "Pathology" - edited by W. Anderson, C. V. Mosby Co. 1948.

50. Henderson, J.: A Fourth Type of Erythroblastosis Fetalis showing Hepatic Cirrhosis in the Macerated Fetus, Arch. Dis. Child. 17: 49, March 1942.

51. Harrison, C. V. and Meacock, E. C.: Rhesus Test: Value in Obstetrics, J. Obst. and Gynec. Brit. Emp. 52: 36 February 1945.

52. Hawksley, J. C. and Lightwood, R.: A Contribution to the Study of Erythroblastosis: Icterus Gravis Neonatorum, Quart. J. Med. 3: 155, April 1934.

53. Philpott, N. W., Hendelman, M., Primrose, T.: The Use of Methionine in Obstetrics, Am. J. Obst. and Gynec. 57: 125, 1949.

54. Drummond, R. J. and Watkins, A. C.: The Rh Factor and Hepatomegaly and Splenomegaly in Children and Adolescents, Brit. Med. J. 1: 984, 1946.

55. Miller, L. L., Rogers, C. A., Lamson, B. G., and Lambie, M. W.: Protein Depletion and Hepatic Function in the Dog. Rationale of Methionine in Therapy, Am. J. Med. Sc. 214: 84, 1947.

56. Sims, F. H.: The Metabolism of Methionine and Choline in Normal and Pre-eclamptic Pregnant Women, J. Obst. and Gynec. of Brit. Emp. 58: 6, December 1951.