16 passages intraspinally in mice and has continued to have an incubation period of 5 to 6 days. Mouse passage 15 cords were inoculated intracerebrally into 2 monkeys (K80 and K81) in a 20% emulsion. Both monkeys developed fever, marked tremors and hind leg paralysis starting on the 7th day.

*Discussion and summary.* Leon type of human poliomyelitis virus has been adapted to mice from original monkey cord virus by use of an intraspinal technic of inoculation. This mouse adapted Leon virus has been serially passed through mice for 35 passages and causes flaccid paralysis, more frequently in the front legs, after an incubation period of 4 to 6 days. A second similar adaptation has been accomplished and this strain carried through 16 mouse passages. The mouse virus causes paralysis only after intraspinal inoculation of mice, being negative when given intracerebrally and is of low virulence thus far. Apparently this virus is still as virulent for monkeys as it is for mice. Proof that this mouse virus is indeed Leon poliomyelitis virus rests on its ability to cause typical clinical and histopathological disease after intracerebral inoculation of monkeys, cross immunization against monkey Leon virus in monkeys and cross neutralization tests in mice and in monkeys. The virus also fails to propagate in the chick embryo. It is neutralized by human gamma globulin in low dilutions. Thus far attempts to adapt the Brunhilde type of human poliomyelitis virus to mice by this same method have been completely negative.

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## Megaloblastic Anemia in Pregnancy. Remission Following Combined Therapy with Ascorbic Acid and Vitamin $B_{12}$.* (19033)

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A recent report by Thompson and Ungley (1) has reviewed many of the clinical and hematologic features of megaloblastic anemia in pregnancy. These authors emphasize the lack of response to vit. $B_{12}$. Similar observations had previously been reported by Day, Hall and Pease(2). In this respect the megaloblastic anemia in pregnancy is similar to megaloblastic anemia experimentally produced in monkeys(3) and to the megaloblastic anemia in some infants(4). Remissions are regularly produced in these three as well as other megaloblastic anemias by folic acid therapy. The megaloblastic anemia in some infants and as experimentally produced in monkeys is related to a deficiency in ascorbic acid and can be prevented by a normal intake of this substance. May(3) has suggested that the ascorbic acid deficiency may interfere with the conversion of folic acid to the citrovorum factor as Nichol and Welch(5) have shown that ascorbic acid augments the conversion of folic acid to the citrovorum factor *in vitro*. It is entirely possible that megaloblastic anemia in some infants and that produced in monkeys results from a deficiency of the citrovorum factor which in turn is produced by altered folic acid metabolism resulting from ascorbic acid deficiency.

Observations on 5 patients with megalo-

* This study was carried out under a grant from Eli Lilly and Co.

1. Thompson, R. B., and Ungley, C. C., *Quart. J. Med.*, 1951, v20, 187.

2. Day, L. A., Hall, B. E., and Pease, G. L., *Mayo Clinic Staff Meetings Proc.*, 1949, v24, 7.

3. May, C. D., Nelson, E. W., Lowe, C. U., and Salmon, R. J., *Am. J. Dis. of Child.*, 1950, v80, 191.

4. May, C. D., Nelson, E. W., Aldrich, R. A., Salmon, R. J., and Lienke, R. I., *Am. J. Dis. of Child.*, 1949, v77, 127.

5. Nichol, C. A., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 52.

blastic anemia in pregnancy indicate that a similar mechanism may hold for the pathogenesis of this anemia. Megaloblastic anemia in pregnancy is refractory to vit. $B_{12}$. Two of the cases to be reported confirm this finding. The pregnancy megaloblastic anemia may be related to ascorbic acid deficiency. A seasonal incidence was present in that all 5 cases appeared in April and May. Lund (6) has shown decreased plasma ascorbic acid values in normal pregnancy during winter and spring months when exogenous sources of ascorbic acid are apt to be low. In 3 of the patients on whom determinations for plasma ascorbic acid were run, decreased or zero values were obtained. To test the analogy further, 3 of the patients were treated with vit. $B_{12}$ and ascorbic acid separately and then in combination. Combined therapy in each of the 3 patients produced a complete hematologic remission where separate therapy with ascorbic acid or vit. $B_{12}$ was ineffective. A fourth patient was first seen after 10 days therapy with vitamin $B_{12}$ during which time the anemia had become progressively more marked. This patient responded to combined folic acid and transfusion therapy. No therapy was given the fifth patient. A spontaneous remission followed delivery. The diagnosis of megaloblastic anemia was made in each case by bone marrow biopsy.

Case 1. D.H. Age 23 Para 2-0-0-2. The diagnosis of megaloblastic anemia was made 7 days before delivery. The hemoglobin was 8.8 g %, erythrocytes 2.66 million, hematocrit 23% and reticulocytes 1.2%. Free acid was present on gastric analysis. The plasma ascorbic acid level was 0.3 mg %. Daily administration of 500 mg of ascorbic acid by intramuscular injection produced a maximum reticulocyte response of 3.6% on the fifth day. The marrow was unchanged at the completion of this test period. While on ascorbic acid therapy the hemoglobin dropped to 6.2 g %, erythrocytes to 1.76 million and the hematocrit to 18%. A daily dose of 30 $\mu$g of vit. $B_{12}$ was added to the ascorbic acid therapy and was followed by a maximum reticulocyte response of 25.4% 9 days after the onset of combined therapy. The marrow reverted to normoblastic development. Rapid clinical and hematologic improvement followed. On discharge from the hospital 27 days post partum the hemoglobin was 11.6 g %, erythrocytes 2.93 million and hematocrit 33%. Later checks showed a return to normal hematologic values.

Case 2. D.H. Age 24 Para 3-0-0-3 (Same patient as case 3). The patient was followed throughout this pregnancy. A diagnosis of megaloblastic anemia was made in the ninth month of pregnancy. Hemoglobin was 8.1 g %, erythrocytes 2.54 million, hematocrit 23% and reticulocytes 0.6%. After the initial workup, daily administration of 500 mg of ascorbic acid was started. A trial period of 4 days was allowed at the end of which time the marrow was still megaloblastic. No reticulocyte increase was observed. The hemoglobin had dropped to 6.4 g %, erythrocytes to 1.86 million and hematocrit to 20%. Thirty micrograms of intramuscular vitamin $B_{12}$ was combined with the ascorbic acid and 9 days later a maximum reticulocyte response of 15.4% was noted. Marrow reversion to normoblastic pattern was noted after the institution of combined therapy. Clinical and hematologic response again was rapid. Twenty-eight days after delivery the hemoglobin was 11 g %, erythrocytes 3.72 million and hematocrit 37%.

Case 3. M.S. Age 18 Para 0-0-0-0. Megaloblastic anemia was diagnosed in the eighth month of pregnancy. Plasma ascorbic acid level was zero. Hemoglobin was 8.6 g %, erythrocytes 2.14 million, hematocrit 25% and reticulocytes 0.4%. Treatment with 30 $\mu$g of intramuscular vit. $B_{12}$ over a 6-day period did not produce a reticulocyte response. At the end of this 6-day period the bone marrow was unchanged. The hemoglobin dropped to 7.6 g %. The combination of 300 mg of ascorbic acid with vit. $B_{12}$ was followed in 4 days by an increase in reticulocytes to 13.4%. Seventy-two hours after the onset of combined therapy the bone marrow was normoblastic. Twenty days after admission and while still undelivered the hemoglobin was 10.4 g %, erythrocytes 2.67 million

6. Lund, C. J., and Kimble, M. S., *Am. J. Obstet. and Gynecol.*, 1943, v46, 635.

TABLE I. Summary of Data on 5 Cases of Megaloblastic Anemia in Pregnancy.

| | Date | Comment | Hemoglobin, g % | Erythrocytes, million $\mu^3$ | Hematocrit, % | Reticulocytes, % |
|---|---|---|---|---|---|---|
| D.H. | 2-28 | Initial workup | 8.8 | 2.66 | 23 | 1.2 |
| | 3-7 | Day of delivery | 7.4 | 1.75 | | 1.3 |
| | -8 | Ascorbic acid therapy begun | | | | |
| | -13 | | 6.2 | 1.76 | 18 | 3.5 |
| | -14 | $B_{12}$ therapy started | | | | |
| | -23 | Max retic response | 7.5 | 2.11 | 24 | 25.4 |
| | 4-3 | Discharge | 11.6 | 2.93 | 33 | 3.4 |
| D.H. | 1-12 | 6th month of pregnancy | 12.5 | 3.35 | 36 | 1.2 |
| | 4-12 | Initial workup | 8.1 | 2.54 | 23 | .6 |
| | -17 | Ascorbic acid therapy begun | | | | |
| | -18 | Day of delivery | | | | |
| | -20 | | 7.1 | 1.86 | 20 | 1 |
| | -21 | $B_{12}$ therapy started | | | | |
| | -30 | Max retic response | 8.6 | 2.23 | 29 | 15.4 |
| | 5-16 | Discharge | 11.1 | 3.72 | 37 | 2.8 |
| M.S. | 5-9 | 8th month of pregnancy | 8.6 | 2.14 | 25 | .4 |
| | -15 | Vit. $B_{12}$ therapy started | 7.6 | | | |
| | -21 | Ascorbic acid therapy started | | | | |
| | -25 | Max retic response | 10.7 | | | 13.4 |
| | 6-18 | Day of delivery | 13.5 | 3.05 | 37 | 1.8 |
| I.M. | 5-4 | Admitted to hospital | 5.1 | 1.41 | | |
| | -6 | Crude liver and $B_{12}$ started | | | | |
| | -12 | Transfusion 500 cc | | | | |
| | -13 | Day of delivery, folic acid | 6.9 | 1.53 | 15 | .3 |
| | -21 | Transfusion 500 cc | 11 | 3.16 | | |
| S.K. | 5-12 | Day of delivery | | | | |
| | -13 | Initial workup | 8.4 | 2.83 | 27.5 | .2 |
| | -23 | Max retic response | | | | 5.5 |
| | 6-24 | | 11.5 | 3.38 | 39 | .6 |

and hematocrit 30%. At delivery 6 weeks after admission the hemoglobin was 13.5 g % and hematocrit 37%.

Case 4. I.M. Age 34 Para 1-0-2-1. Megaloblastic anemia was diagnosed on the day of delivery though the patient had been hospitalized and treated with vit. $B_{12}$ and crude liver extract for 10 days prior to diagnosis. Following delivery an adequate response was obtained with folic acid and blood transfusions. The initial hemoglobin was 6.9 g %, erythrocytes 1.53 million, hematocrit 15% and reticulocytes 0.3%. Aside from the demonstration that vit. $B_{12}$ administration had not prevented the appearance of this megaloblastic anemia, little information was obtained.

Case 5. S.K. Age 42 Para 7-2-2-9. The diagnosis of megaloblastic anemia was made on the day following delivery. The hemoglobin was 8.4 g %, erythrocytes 2.83 million, hematocrit 27.5% and reticulocytes 0.2%. There was free acid on gastric analysis. Plasma ascorbic acid value was zero. Inadvertently no therapy was given this patient but a spontaneous remission followed delivery. A maximum reticulocyte response of 5.4% appeared 11 days after delivery. When seen 43 days after delivery the hemoglobin was 11.5 g %.

The essential data are summarized in Table I. Other tests were made on these patients which are of some interest. In 4 patients on whom stool specimens were obtained before therapy there was no increase in urobilinogen excretion. Serum iron determinations made prior to therapy on 4 patients showed values of 318, 348, 365 and 268 $\gamma$ %. The iron binding capacity of the serum was less than 50 $\gamma$ % in the same 4 patients. Following combined therapy there was a sudden decrease in serum iron values to less than 50 $\gamma$ % and increases in the iron binding capacity. Normal erythrocyte protoporphyrin values were obtained on all 5 patients prior to therapy. After therapy these values increased

as the bone marrow reverted to normoblastic activity.

It is possible that ascorbic acid therapy alone over a long enough period of time would produce a remission. In Case 1 a suggestive increase in reticulocytes occurred but therapy was not continued long enough to warrant any conclusions. It is probable that megaloblastic anemia in pregnancy can be prevented by adequate ascorbic acid intake during pregnancy.

*Summary.* Five cases of megaloblastic anemia in pregnancy are reported. The relation of this anemia to a deficiency in ascorbic acid is suggested by the effectiveness of combined ascorbic acid and vit. $B_{12}$ therapy where vit. $B_{12}$ alone was ineffective. Two patients were refractory to adequate trial with vit. $B_{12}$. One of these patients later had a complete remission when ascorbic acid was added to the therapy. The other responded adequately to folic acid therapy and blood transfusions. Two patients were treated for short periods of time with ascorbic acid alone. A minimal response in reticulocytes was observed in one. Subsequent addition of vit. $B_{12}$ to the therapy after saturation with ascorbic acid produced a complete hematologic remission. One patient had a spontaneous remission after delivery. In 3 patients combined therapy with ascorbic acid and vit. $B_{12}$ produced a complete remission.

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## Fiber Formation in Trypsinogen Solutions: An Electron Optical Study.* (19034)

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Recently the author(1) reported a study of the structure of elastic tissue with the electron microscope which emphasized a seemingly specific effect of trypsin on elastic fibers in releasing large numbers of characteristically coiled threads. These were composed of pairs of smooth filaments each about 70 A in diameter coiled in a tight, regular helix with a pitch of 500 A and a width of 120 A. Numerous unwound filaments were also observed. It was also reported that electron micrographs of freshly prepared trypsin solutions dried on the supporting film did not show the coiled threads. More recently Franchi and DeRobertis(2) reported the appearance of coiled threads, resembling those described above, in solutions of Armour's crystalline trypsin after incubation—in the absence of elastin—whereas, after sterilization of the trypsin solution by Seitz filtration, no such elements were found. They also reported fresh trypsin solutions to be devoid of fibers. Because a few bacteria and flagella were found in the non-sterilized preparations, they suggested that the coiled threads observed by Gross and themselves were degradation products of tryptically digested flagellae. In a second paper(3) these authors described the appearance of coiled threads in a culture of *Bacillus brevis* incubated in Armour's crystalline trypsin. This organism was found as a contaminant of the enzyme.

This paper describes a reinvestigation of the

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1. Gross, J., *J. Exp. Med.*, 1949, v89, 699.

2. Franchi, C. M., and DeRobertis, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 515.

3. DeRobertis, E., and Franchi, C. M., *Exp. Cell. Res.*, 1951, v2, 295.