THE ERYTHROPOIETIC ACTIVITY OF CHOLINE CHLORIDE IN MEGALOBLASTIC ANEMIAS

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INTRODUCTION

MOOSNICK, Schleicher, and Peterson reported in 1945 that the administration of choline chloride resulted in the hematological remission of a case of pernicious anemia refractory to parenteral liver therapy. This action, attributed to choline, appeared to merit further study since, if it were confirmed, it would be desirable not only to assess the value of choline in the therapy of anemia but also to explore its possibilities in the elucidation of the etiology of megaloblastic anemias, especially those refractory to parenteral liver therapy. The present investigation was therefore undertaken with the object of observing the erythropoietic activity of choline in various types of megaloblastic anemia.

Before describing our findings, it may not be out of place to recapitulate the observations of Moosnick et al. Their patient, a man aged 61 years, had been successfully treated for several years with intramuscular injections of liver extract but eventually relapsed, despite high dosage of refined liver extracts, and developed sensitivity to liver extracts, both refined and crude. When admitted to the hospital he was suffering from severe jaundice. The red cell count was 3.87 M., the sternal marrow showed megaloblastic erythropoiesis but was hypoplastic with fatty metamorphosis. Liver biopsy showed acute hepatitis and moderately severe fatty changes.

Choline chloride was administered intravenously in a daily dose of 1 gram for 16 days and was followed by a reticulocyte response of 5.5 per cent on the third day and a progressive rise in the red cell count. At the end of the period of treatment the peripheral blood status was normal, the patient's general condition improved, and the sternal marrow picture restored toward normality. The patient was subsequently treated with ventriculin but died of pneumonia some months later, at which time autopsy examination revealed that the liver was practically normal.

The authors suggest that in their patient an adequate amount of hematopoietin was stored in the liver but not elaborated in sufficient amounts on account of the fatty state of this organ and that this dysfunction was corrected by the administration of choline. Although there was no direct evidence of choline deficiency, it was thought that this was present on account of the conspicuous fatty changes in the liver and sternal marrow.

Although the case recorded by Moosnick et al. seems to be exceptional in that...
it was complicated by severe hepatic disease, it appeared to us that, if their hypothesis were correct, the refractoriness to parenteral liver therapy displayed by certain other types of megaloblastic anemia might also result from hepatic dysfunction amenable to choline therapy. It also seemed possible, in view of the effect of choline on intestinal absorption (Frazer2), that choline might exert a favorable influence on megaloblastic anemias associated with defective intestinal function.

METHODS

The clinical material studied by us consisted of io cases of macrocytic anemia, in all of which erythropoiesis, as revealed by sternal puncture, was megaloblastic. The cases are considered to be representative of five types of megaloblastic anemia, namely: (i) Addisonian pernicious anemia, (2) megaloblastic anemia of pregnancy, (3) megaloblastic anemia of the sprue syndrome, (4) nutritional megaloblastic anemia, and (5) refractory megaloblastic anemia of uncertain etiology.

The choline chloride employed was a commercial preparation (British Drug Houses) and was administered either orally dissolved in water or intravenously dissolved without previous heating in sterile normal saline. The daily dosage varied from 3 to 10 grams when given orally and from 1 to 10 grams when given intravenously. No side effects accompanied the oral medication. Unpleasant reactions were, however, apt to result from the intravenous administration of choline. When the dose was limited to 1 gram given slowly in a 5 per cent solution, these reactions were minimal, but they constituted a major objection to the giving of large doses. Doses of 5 or 10 grams were given in a 1 per cent slow drip infusion over periods exceeding three hours but were usually accompanied by unpleasant manifestations such as tachycardia, nausea, vomiting, sweating, abdominal discomfort, and depression. No significant reduction in blood pressure was noted, however. On account of these effects it was not feasible to continue high dosage intravenous therapy for more than three or four days.

The liver extracts were from batches known to be potent in cases of Addisonian pernicious anemia. The proteolyzed liver was the commercial preparation, "hepamino," given by mouth in divided doses, totaling approximately 36 grams daily. The oral liver extract was Armour's or Evan's preparation given in doses of 45 or 60 cc. daily.

The food, during the periods of observation, consisted of ordinary hospital diets containing relatively small quantities of first-class protein and no vitamin supplements.

The hematological technic calls for no special description other than to remark that the hemoglobinometers were standardized so that 100 per cent was equivalent to 14.8 grams of hemoglobin per 100 cc. The sternal puncture procedure was as described by Davis, Davidson, and Innes. The liver biopsies were performed by the needle method (Sherlock4).

Case Histories

A. ADDISONIAN PERNICIOUS ANEMIA

Case 1. Mrs. C., aged 71, was admitted to the hospital on August 11, 1946, with a history of symptoms attributable to anemia which had become progressively disabling during the previous three months.
The earlier medical history is not significant. She was a well-nourished woman displaying marked pallor and a slight degree of jaundice. The tongue was pale and slightly atrophied. No enlargement of the liver, spleen or lymph glands was detected and no signs of neurological complications were evident.

Laboratory findings: R.B.C., 1.48 M.; hemoglobin, 40 per cent; color index, 1.3; M.C.V., 117 cu. microns; reticulocytes less than 1 per cent; W.B.C., 1,800 per cu. mm. Stained blood film: typical macrocytic picture with poikilocytosis and anisocytosis. Sternal marrow megaloblastic erythropoiesis, hypercellular with no excessive fat. Gastric analysis: histamine-fast achlorhydria. Liver biopsy: no obvious histological abnormality, glycogen content of cells was normal and there were no gross fatty changes. Urine: urobilinogen was present.

Treatment and progress: Choline chloride in daily doses of 10 grams was given intravenously for three days but had to be discontinued on account of severe side effects. During the following eight days choline was given orally in divided doses aggregating 10 grams daily. On the fourth day after commencing choline therapy the reticulocyte count rose to a maximum of 1.5 per cent, but was not accompanied by any rise in the red cell count which, on the contrary, fell to under 1 million on the thirteenth day. This fall was accompanied by a deterioration in the patient’s general condition requiring a blood transfusion. The patient was subsequently given refined liver extract ("neo-hepatex") parenterally, to which she responded satisfactorily, as shown in figure 1, with subsequent maintenance of a normal blood picture.

Case 2. Miss D., aged 45, was diagnosed as a case of Addisonian pernicious anemia in 1944 and treated with parenteral liver therapy to which she responded satisfactorily. Later, however, she failed to continue treatment and was admitted to the hospital on February 19, 1946, in a severely anemic state. No noteworthy clinical findings were present other than those of severe anemia and a very slight degree of icterus. No enlargement of the liver, spleen, or lymph glands was detected and no signs of neurological involvement were present.

Laboratory findings: R.B.C., 0.7 M.; hemoglobin, 20 per cent; color index, 1.4; M.C.V., 143 cu. microns; reticulocytes, less than 1 per cent; W.B.C., 1,800 per cu. mm. Blood film: macrocytosis, poikilocytosis, and marked anisocytosis. Sternal marrow: megaloblastic, hypercellular, no excess of fat. Gastric analysis: histamine-fast achlorhydria. Urine: urobilinogen present. Liver biopsy was not performed.
Treatment and progress: Intramuscular injections of a refined liver extract ("anahaemin"), totaling 12 cc. in twelve days, resulted in a suboptimal reticulocyte response but in a sustained rise in the red cell count (fig. 2), until a level of 3,100,000 red cells was reached. Thereafter the red cells remained stationary for seven days and then fell to 2.7 M. Choline was then given intravenously in a daily dose of 1 gram for eleven days and was accompanied by a rise in the red cell count to 4.05 M. (fig. 2).

The patient has since been maintained satisfactorily with parenteral liver therapy.

Case 3. Mrs. M. S., aged 63, first developed anemia at the age of 59 and was given injections of liver extract with symptomatic improvement. Since this treatment was not maintained regularly, she was never completely free from symptoms and suffered constantly from a feeling of weakness and from undue dyspnea on effort. During the month prior to admission these symptoms became more severe.

On admission to hospital on November 8, 1946, she displayed the usual manifestations of severe anemia. Jaundice was absent, however, and no enlargement of liver, spleen, or lymph glands was detected, nor was there evidence of neurological disease.

Laboratory findings: R.B.C., 1.3; hemoglobin, 40 per cent; color index, 1.5; W.B.C., 4,600 per cu. mm.; reticulocytes, less than 1 per cent. Stained films showed marked macrocytosis, anisocytosis, and poikilocytosis. Sternal marrow: megaloblastic, hypercellular with no excess of fat. Gastric analysis: histamine-fast achlorhydria. Liver histology: a biopsy performed twenty days after the first injection of liver extract and nine days before administration of choline showed no abnormalities other than a slight degree of patchy fatty degeneration. A second biopsy seventeen days after the institution of choline therapy showed no fatty change or other abnormality. The serum colloidal gold and the plasma alkaline phosphatase were within normal limits shortly after admission to hospital.

Treatment and progress: After an observation period of five days a single injection of liver extract ("hepastab" 4 cc.) was followed by a reticulocyte response of 18 per cent and a rise in the red cell count to 3.02 M on the twenty-fourth day. Thereafter the red cells fell to 2.75 M. on the thirtieth day. Further injections of liver extract were deliberately withheld in order to provide an opportunity of observing the effect of choline. Choline chloride was given intravenously in daily doses of 1 gram for twelve days and then by mouth in doses of 15 grams daily for a further period of fourteen days. Reference to figure 3 will show that the administration of choline was accompanied by a rise in the red cell count to 3.7 M.
although no significant reticulocyte response was observed. The regeneration of red cells was not main-
tained, however, despite the continuation of oral choline therapy, and eventually, after withdrawal of
choline, the red cells fell to 2.9 M. Parenteral liver therapy was then resumed with a consequent reticulo-
cyte response of 7 per cent and a rise in the red cell count which eventually attained a normal level.

COMMENT ON CASES 1, 2, AND 3

In case 1, an example of untreated Addisonian pernicious anemia, choline was
without significant effect. It will be noted that the substance was given in daily
doses of 10 grams intravenously for three days and orally for seven more days.
Subsequent parenteral liver therapy resulted in satisfactory red cell regeneration,
although the reticulocyte response was suboptimal.

In cases 2 and 3, treatment was initiated by injections of liver extract which
resulted in regeneration of red cells although the reticulocyte responses were sub-
optimal. When the hematopoietic effect of the liver extracts was apparently ex-
hausted and the red cell count was falling, the administration of intravenous
choline in both cases resulted in a further rise in the red cell count. Although no
reticulocyte response to choline was observed in either case, it is reasonable to
assume that the red cell response is attributable to the administration of choline.*

* Since submitting this paper for publication, we have observed the effect of choline chloride in 3
further cases of classical Addisonian pernicious anemia. In each case the choline was given subsequent to
an initial hemopoietic response to a single injection of liver extract, after the red cell count had commenced
to fall. The dosage was 1 gram daily for a period of five to seven days; the route was intravenous.

One case showed no significant response, but in the other 2 cases the administration of choline was
followed by secondary but temporary rises in the red cell counts and hemoglobin levels similar to that
described in cases 2 and 3 in the text. The magnitude of these secondary rises was from 1.6 M. to 2.95
M. in the one case and from 1.87 M. to 3.51 M. in the other.
It is noteworthy that in case 3 choline by mouth was not effective in maintaining a continued rise in the red cells.

B. megaloblastic anemia of pregnancy

Case 4. Mrs. D. D., aged 36, developed a severe anemia during the later stages of pregnancy. She received injections of liver extract without effect and blood transfusions before and after a premature delivery. During the three months immediately following her delivery numerous injections of liver extracts (anahaemin) resulted in no improvement in her anemia. At this time, April 15, 1946, she came under our care. She was a pale, somewhat poorly developed woman but not ill nourished. Apart from manifestations of anemia, no noteworthy clinical findings were present. There was no evidence of jaundice.

Laboratory findings: R.B.C., 1.4 M.; hemoglobin, 35 per cent; color index, 1.25; M.C.V., 115 cu. microns; W.B.C., 4,600 per cu. mm.; reticulocytes, less than 1 per cent. Blood film: macrocytic, hyperchromic. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: free hydrochloric acid present in resting juice. Liver biopsy was not performed.

Treatment and progress: In view of the history, this case was diagnosed as a refractory megaloblastic anemia of pregnancy and puerperium, but to confirm the refractoriness to parenteral liver extract, an injection of anahaemin (4 cc.) was given. As shown in figure 4, this was without hemopoietic effect. The patient was then given choline—10 grams daily by mouth for ten days, followed by intravenous injections of 1 gram for a further period of seven days. This was followed by no reticulocyte response, while the red cell count fell. Oral liver extract was then given and resulted in a reticulocyte response of 17 per cent and a rapid rise in the red cell count from 1.05 M. to 3.64 M. in twenty-five days. Subsequently the count rose to 4.8 M. It remained at approximately this level during the following three months without further treatment.

Case 5. Mrs. M., aged 36, developed anemia during the latter half of her sixth pregnancy and was treated with iron. She was delivered of a healthy child but since the signs of anemia became more severe, a sternal puncture was performed seventeen days after delivery and an injection of liver extract (anahaemin, 4 cc.) was given on the same day. She came under our care six days later, on April 1, 1946.
Physical examination revealed no significant features other than those of anemia. Jaundice was not present and enlargement of liver, spleen or lymph glands was not demonstrated. Laboratory findings: R.B.C., 1.56 M.; hemoglobin, 36 per cent; color index, 1.16; M.C.V., 109 cu. microns; reticulocytes, 4.5 per cent; W.B.C., 4,000 per cu. mm.; blood film, macrocytic. Sternal marrow: The films prepared on the occasion already mentioned were subsequently seen by us and showed megaloblastic erythropoiesis. Gastric analysis: free hydrochloric acid in resting juice.

Treatment and progress: A further injection of liver extract ("perhepar," 4 cc.) was followed by a delayed and suboptimal reticulocyte response, but the red cells showed a sustained rise, attaining a level of 3.2 M. by the twenty-fifth day, and then remained stationary at this level in the absence of further treatment for seventeen days. Choline chloride was then given intravenously in daily doses of 1 gram for ten days. A reticulocyte response of 3.5 per cent followed and a further rise in the red cell count occurred, reaching a level of 4.2 M., a month after commencing choline therapy (figure 5). No subsequent treatment was given and eventually the blood picture reached and remained at normality.

Comment on Cases 4 and 5

Both these cases are clearly examples of megaloblastic anemia of pregnancy, as described by Davidson, Davis and Innes. Case 4 was refractory to intensive parenteral liver therapy but subsequently responded to oral liver preparations. Choline, in the dosage employed, appeared to be completely lacking in effect in spite of numerous recent injections of liver extract. It would therefore seem in this case that the lacking erythropoietic factor, which parenteral liver therapy was unable to supply, was not choline or any agent which could be made available by choline.

Case 5 was an example of a common type of megaloblastic anemia of pregnancy which responds to parenteral liver therapy in the puerperium. As is well known, such cases frequently undergo spontaneous remission during the months following birth of the child. We therefore are of the opinion that it would be exceedingly
rash to conclude that the choline given in this case was necessarily responsible for the subsequently slow rise in the red cell count. The slight reticulocyte count, however, does appear to be suggestive that the choline may have exerted some erythropoietic effect. It should be noted that this apparent response to choline followed an earlier response to liver extracts.

C. MEgaloblastic Anemia Associated with the Sprue Syndrome

Case 6. Miss S., aged 16, had suffered from celiac disease since infancy and came under our care in April 1946. She was an underdeveloped and poorly nourished girl, very small for her age. Many of the features of idiopathic steatorrhea were present, including osteoporosis, papillary atrophy of the tongue, slight clubbing of the fingers and the constant passage of bulky pale stools containing excess fat. The usual signs of anemia were present. There was no evidence of jaundice or of enlargement of the liver, spleen, or lymph glands.

Laboratory findings: R.B.C., 1.5 M.; hemoglobin, 25 per cent; color index, 0.8; M.C.V., 100 cu. microns; M.C.H.C., 23 per cent; reticulocytes, 7 per cent; W.B.C., 8,500 per cu. mm. Blood films showed a "dimorphic" macrocytic, hypochromic picture. Sternal marrow: erythropoiesis was partly megaloblastic, many undoubted megaloblasts were present but the early megaloblasts were relatively small and normoblasts were relatively numerous. Gastric analysis: histamine-fast achlorhydria.

Treatment and progress: An injection of refined liver extract (anaehaemin, 4 cc.) was followed by only a slight reticulocyte response and no sustained increase in the red cells (figure 6). Because of the patient's poor veins, repeated intravenous injections were not feasible and therefore it was decided to try the effect of choline by mouth; 105 grams of choline chloride given during 14 days resulted in no observable effect. Oral liver extract was then given and was followed by a brisk erythropoietic response, the reticulocytes rising to 17 per cent and the red cells from 2.0 to 3.4 M. within twenty-four days. After her discharge from the hospital she continued to take oral liver extract and when last seen the red cell count was 3.18 M. and her general condition improved.

Case 7. Miss P., aged 25, was apparently healthy until the age of 18 when she became anemic and was treated with iron and injections of liver extract. The injections were maintained, somewhat ir-
regularly, however, for seven years, when in spite of increased dosage her general condition deteriorated and the passage of loose pale stools was first noted. She was then investigated more fully in an outpatient clinic, when the diagnosis was established of idiopathic steatorrhea and of associated macrocytic anemia. At this time (November 1945) the salient laboratory findings were as follows: R.B.C., 2.33 M.; hemoglobin, 55 per cent; color index, 1.18; M.C.V., 107 cu. microns; M.C.H.C., 32 per cent; W.B.C., 8,900 per cu. mm.; plasma bilirubin, 0.5 mg. per cent; gastric analysis: free hydrochloric acid in fasting juice; fecal fat: 38 per cent of dried weight; glucose tolerance curve: flat; x-ray: skeletal osteoporosis.

The patient was subsequently kept under observation and on March 21, 1946, she developed a severe relapse necessitating her admission to hospital. Clinical examination revealed a short (5 feet) but relatively well nourished woman with no evident pathological signs other than those of severe anemia.

Laboratory findings: R.B.C., 1.2 M.; hemoglobin, 24 per cent; color index, 1.0; M.C.V., 101 cu. microns; M.C.H.C., 16 per cent; reticulocytes, less than 1 per cent; W.B.C., 3,000 per cu. mm.; sternal marrow, megaloblastic, hypercellular with no excess of fat.

![Fig. 7. A case of megaloblastic anemia associated with the sprue syndrome. No response occurred to an injection of liver extract followed by oral choline, but the administration of oral liver extract resulted in the restoration of a normal blood picture.](image)

Treatment and progress: In view of her condition, she received a blood transfusion of 500 cc. of whole blood followed by 500 cc. of packed cells, which raised the red cell count to 2.7 M. Six days later an injection of liver extract (anahaemin, 4 cc.) was administered, but the red cell count fell to 1.54 M. in ten days. At this point choline chloride was given by mouth in daily doses of 10 grams, the oral route being chosen on account of the poor state of the patient's veins. Since no reticulocyte response occurred and the red cell count continued to fall, the choline was discontinued after six days and a further blood transfusion was given. Oral liver extract was then administered and, as seen in figure 7, resulted in a significant reticulocyte response and a progressive rise in the red cells to a level of 4.51 M., which has since been maintained for over a year, the patient continuing to take oral liver extract.

**COMMENT ON CASES 6 AND 7**

Although the investigation of these cases was not so complete as we would have desired, particularly in respect to fat absorption studies, there seems little reason to doubt that the megaloblastic anemia present was associated with an underlying defect in intestinal absorption. It is evident that in neither case could any erythro-
poietic effect be attributed to choline. It is unfortunate that in both cases the choline was given only by the oral route because, for reasons to be discussed later, it is probable that choline is less effective when administered by this route than when given intravenously.

It should be noted that in both cases the administration of choline was preceded by injections of liver extract, which were ineffective, and succeeded by oral liver therapy which was surprisingly efficacious hematopoietically.

D. NUTRITIONAL MEGALOBLASTIC ANEMIA

Case 8. Mrs. A., aged 34, developed symptoms of anemia at the age of 38 and was subsequently treated intermittently with injections of liver extract. She came under our care on August 9, 1946. It is significant that she gave a history of an inadequate diet for some years, especially in respect of meat and vegetables. Clinical examination revealed, in addition to the usual manifestations of anemia, a dry brown pigmentation of the skin over the neck and backs of the hands. The tongue showed a slight degree of papillary atrophy and glazing of the tip. Her general nutritional state was poor. No jaundice was evident, nor was there enlargement of the liver, spleen, or lymph glands.

Laboratory findings: R.B.C., 1.48 M.; hemoglobin, 44 per cent; color index, 1.5; reticulocytes, less than 1 per cent; W.B.C., 5,400 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: free hydrochloric acid after injection of histamine. Liver biopsy: slight diminution in cellular glycogen and a slight increase in hemosiderin in both the littoral and liver cells, otherwise no abnormalities. Urine: no abnormalities.

Treatment and progress: An injection of liver extract (anahemin, 4 cc.) was followed by a reticulocyte response of 6 per cent on the fifth day, but no significant rise in the red cell count. Choline chloride was administered over a period of ten days commencing on the thirteenth day after the injection of liver extract. As shown in figure 8, the choline was given intravenously 5 grams daily for three days, but on account of severe side reactions this was reduced to 1 gram for the remaining seven days during which time it was supplemented by 10 grams daily by mouth. A second injection of liver extract (ana- hemin, 4 cc.) was given on the sixth day after commencing choline. This was given with the object...
of providing an adequacy of the active principle in liver extract, and of thus testing the possibility that liver extract and choline might be active when given together, although inert when given separately. No such effect was noted, however, apart from a reticulocyte response of only 2 per cent, clearly of very doubtful significance, and the red cell count continued to fall. Three days after stopping the choline therapy, synthetic folic acid was given in daily doses of 20 mg. by mouth, which resulted in a reticulocyte response of 33 per cent on the eighth day and a brisk rise in the red cell count. A second sternal puncture now showed normoblastic erythropoiesis.

Continuation with folic acid therapy eventually resulted in the red cells rising to 4.75 M. This level has subsequently been maintained without further treatment.

COMMENT ON CASE 8

In view of the history and the clinical and laboratory findings, this case is regarded as an example of nutritional megaloblastic anemia refractory to treatment with refined liver extracts administered parenterally. It seems evident that choline chloride, in the doses employed, was without effect. It should be noted that although liver biopsy was performed in this patient, there was no evidence of gross fatty changes, nor were such changes seen in the sternal marrow films. The response to folic acid is of interest. It will be seen that the initial response was optimal, but the subsequent rise in the red cell count became retarded. Similar results with folic acid in the treatment of refractory megaloblastic anemias have been noted by Davidson and Girdwood. In our case, however, it will be seen (fig. 8) that increasing the dose of folic acid was followed by further red cell regeneration.

E. REFRACTORY MEGALOBLASTIC ANEMIA OF UNCERTAIN ORIGIN

Case 9. Mrs. A. B., aged 51. A diagnosis of pernicious anemia had been made four years previously and injections of liver extract had apparently been successful for a time, but failure to continue treatment resulted in relapse. During the autumn of 1945, the patient was given numerous injections of liver extract by her doctor without improvement. She came under our care on January 29, 1946, in a state of moderately severe relapse despite numerous injections of liver extract given during the preceding weeks. The patient was an obese woman displaying the usual manifestations of anemia. No signs of jaundice were present and no enlargement was detected of the liver, spleen, or lymph glands. Signs of neurological disease were absent.

Laboratory findings: R.B.C., 1.88 M.; hemoglobin, 52 per cent; color index, 1.38; reticulocytes, less than 1 per cent; W.B.C., 3,400 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: histamine-fast achlorhydria. Urine: trace of urobilinogen.

Treatment and progress: An injection of liver extract (anaehaemin, 4 cc.) was given but no reticulocyte response occurred within six days and the red cell count continued to fall. The oral administration of choline was then commenced and continued for three weeks, the dose being 3 grams daily. The following day the reticulocyte count was 4 per cent and rose to a maximum of 22 per cent on the ninth day after commencing choline and the fifteenth day after the injection of liver extract. It will be seen in figure 9 that the reticulocytes did not immediately return to a low level but continued at about 5 per cent during, and for a short time after, the first period of choline therapy. It will also be seen that the red cell count slowly rose during this period to a maximum of 2.39 M. A second sternal puncture performed at about this time, however, still showed frankly megaloblastic erythropoiesis. On the forty-second day after the first injection of liver extract and after the reticulocyte count had again fallen to less than 1 per cent, another injection of liver extract was given (anaehaemin, 2 cc.) and was followed by prompt reticulocyte response of 5 per cent but no rise in red cells. Thirteen days later, when the reticulocytes had again fallen to less than 1 per cent, the injection of liver extract was repeated (anaehaemin, 2 cc.) and oral choline therapy recommenced in the same dosage as before. A similar reticulocyte response occurred, but again without a significant red cell increase. After two weeks of oral therapy the choline was given intravenously in daily doses of 1 gram and continued for ten days. This was accompanied by a reticulocyte response of 11 per cent and a significant rise in the red cell count which eventually reached 3.8 M. without
Fig. 9. A case of megaloblastic anemia resembling Addisonian pernicious anemia which had become refractory to numerous injections of liver extract. The chart illustrates the responses to oral and intravenous choline. For details see text.
further treatment. A further course of oral choline, the dose now being 10 grams daily, was without effect on the reticulocytes. The red cells continued to rise to 4.15 M. but subsequently fell during the last period of therapy. Proteolyzed liver (36 grams daily by mouth) was then given and resulted in the eventual restoration of the red cell count to 5 M. The patient’s general and hematological condition has since remained satisfactory on maintenance therapy with proteolyzed liver.

Case 10. Mrs. S. B., aged 52, had been healthy until September 1946, when she developed symptoms of anemia. Following a diagnosis of pernicious anemia she received a course of injections of liver extract to which she failed to respond. She was then seen by one of us on December 8, 1946. The usual symptoms and signs of anemia were present and a mild degree of jaundice was noted, but there was no demonstrable enlargement of the liver, spleen, or lymph glands, or other significant pathological features.

Laboratory findings: R.B.C., 1.4 M.; hemoglobin, 31 per cent; color index, 1.1; reticulocytes, less than 1 per cent; white cells, 4,000 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: histamine-fast achlorhydria.

Treatment and progress: The patient had already received frequent injections of liver extract totaling at least 2.6 cc. during the previous three weeks (see fig. 10). This had resulted in no improvement in her red cell count; reticulocyte counts, however, had not been recorded throughout the whole of this period. Choline was given intravenously in daily doses of 1 gram for three days and was followed by a reticuloocyte response of 9 per cent on the fifth day, and a rise in the red cell count from 1.3 M. to 3.4 M. on the seventeenth day. The patient was subsequently treated with injections of liver extract (anaheamin) with consequent restoration and maintenance of a normal blood picture.

COMMENT ON CASES 9 AND 10

The etiological status of case 9 is obscure. A diagnosis of Addisonian pernicious anemia would be justified were it not for the inadequate response to parenteral liver therapy. This case corresponds to a type of idiopathic refractory megaloblastic anemia previously described by Davidson, Davis, and Innes, and Davis and David-
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son and in many respects it is similar to that of Moosnick, Schleicher, and Peterson, although jaundice and gross disturbances of fat metabolism were features of their case. No liver biopsies were performed in our case, but the sternal marrow smears provided no evidence of gross fatty changes as illustrated by these authors. It is unfortunate that the first period of oral choline therapy was commenced so soon after the injection of liver extract, since it is impossible to exclude the effect of the latter in producing the subsequent reticulocyte response. In view, however, of the absence of any reticulocyte response within seven days of the injection of liver extract, it would seem that the choline probably played at least some part in the production of the reticulocyte and red cell response. It must be remembered, furthermore, that numerous injections of liver extract had been given during several weeks immediately preceding the patient's admission to hospital. During the next phase of the experiment two injections of liver extract were given, the second of which was followed by a further course of oral choline. The response to each of these was identical, namely, a slight reticulocyte response but no increase in the red cells. The subsequent period of intravenous choline therapy, without further liver extract was, however, followed by another and greater reticulocyte response and a significant rise in the red cell count. It may be concluded therefore from this case that the choline chloride administered intravenously exerted a significant erythropoietic effect, and that choline by mouth probably produced some effect.

In case 10 the response to choline seems unequivocal. It will be noted that this patient proved refractory to numerous injections of liver extract given shortly before the administration of choline. In view of the patient's subsequent response to further injections of liver extract, it would appear that the earlier refractoriness to parenteral liver extract was a temporary phase. Whether choline played a part in overcoming this refractory phase is, of course, conjectural. We feel that it is particularly unfortunate in this case that no liver biopsy was done before the institution of choline therapy. It is perhaps significant, however, that the sternal marrow material showed no evidence of the fatty changes reported by Moosnick et al.

Discussion

ANALYSIS OF RESULTS

The following conclusions are suggested by our observations.

1. Choline by itself is incapable of rectifying megaloblastic erythropoiesis or of exerting a significant erythropoietic effect. This conclusion is based upon case 1, an example of untreated Addisonian pernicious anemia which showed no response to choline but responded satisfactorily to subsequent injections of liver extract. There are, of course, no theoretical grounds for believing choline to possess intrinsic hematinic activity. On the contrary, choline has been claimed to depress erythropoiesis by its vasodilator action in increasing the oxygenation of the bone marrow (Davis).

2. Choline appears to be capable of exerting a "boosting" effect in cases of megaloblastic anemia in which a response to parenteral liver therapy has already occurred.

This conclusion is based on cases 2, 3, and 5, representing two examples of Addi-
sonian pernicious anemia and one of megaloblastic anemia of pregnancy respectively. It will be recalled that these cases all responded to one or more injections of liver extract and that the administration of choline after the erythrocyte count had ceased to rise, or had actually begun to fall, was followed by a secondary rise in the red cells. Admittedly, a reticulocyte response to choline was noted only in case 5, and in this case the need for caution in its assessment has already been stressed. Nevertheless, in view of the uniform pattern of the erythrocyte response displayed by each of these cases, we believe that it is justifiable to attribute the effect of choline.

3. The effect of choline in megaloblastic anemias refractory to parenteral liver therapy is variable and appears to depend upon the type of case and its underlying etiology. Cases falling under this heading which are included in this study may be divided into two broad groups according to their erythropoietic response to choline.

The first group, consisting of 4 cases all of which showed no response to choline despite intensive parenteral liver therapy immediately preceding its administration, comprise examples of well recognized conditions, namely, anemias associated with pregnancy (case 4), with the sprue syndrome (cases 6 and 7), and with nutritional deficiency (case 8). It is now generally recognised that a considerable proportion of such anemias may show varying degrees of refractoriness to parenteral liver therapy while responding promptly to the administration of liver products by mouth, (Davis and Davidson,6 Davis,9 Fullerton,10 Watson and Castle11). Possible explanations for this phenomenon have been discussed by Davis and Davidson6 and Watson and Castle,11 although recent work on folic acid may lead to a reorientation of the problem (see Davis12). Whatever may be the explanation for the efficacy of oral liver therapy in such cases, our present studies provide evidence that the lack of response to refined liver extracts administered parenterally cannot be corrected by choline. In this connection it is significant that the choline content of proteolyzed liver is approximately 36 mg. per 100 grams (Riding13) which would amount to a daily dosage of only 12 mg. in the dose of proteolyzed liver usually employed. It should be noted that in our two examples of megaloblastic anemia associated with defective intestinal absorption (cases 6 and 7) the choline was given only by the oral route although in large doses. Since it is probable that this method of administration is less effective than the intravenous route, it may be objected that the negative response of these two cases is inconclusive. On the other hand, if choline were capable of exerting an erythropoietic effect in such cases by promoting intestinal absorption (Frazer2) it would seem probable that the oral route would be effective.

The second group consists of the two cases, 9 and 10, classified as refractory megaloblastic anemia of unknown origin. Both these cases would have been regarded as classical examples of Addisonian pernicious anemia had it not been for their ineffectual response to potent parenteral liver extracts. Case 9, in fact, had previously responded to such treatment but subsequently became refractory. In both cases choline, given a short interval after intensive parenteral liver therapy, was followed by a significant erythropoietic response, and in case 10 the patient subse-
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quently became amenable to injections of liver extracts. It seems probable, there-
fore, that in these two cases the nature of the refractoriness to parenteral liver
therapy may differ fundamentally from that of the first group discussed above. It is
perhaps significant that these cases resemble in many respects the case described by
Moosnick et al., although in their case jaundice and severe hepatitis were present.

THE MODE OF ACTION OF CHOLINE

Moosnick et al. suggest that the refractoriness of their patient to parenteral liver
therapy was due to an underlying choline deficiency which resulted in severe hep-
atic disease and disordered fat metabolism, thus rendering the liver incapable of
utilizing or of elaborating the active principle supplied by the liver extracts. Al-
though it is well known that the liver is concerned with the storage of the antiane-
mic principle and occasional cases of megaloblastic anemia have been attributed to
hepatic disease, so far as we are aware there is no conclusive evidence that the
therapeutic activity of liver extracts in pernicious anemia is dependent upon hep-
atic function. In this connection, perhaps it is not irrelevant to refer to recent
work on folic acid, which, if confirmed, may provide an attractive, but at present
totally speculative, theory of the role of the liver in erythropoiesis.

The experiments of Welch et al. suggest that the essential defect in pernicious
anemia is an inability on the part of the organism to liberate free folic acid from
inactive conjugates assimilated from the food, and that the hematopoietic activity
of liver extracts is concerned with the restoration of this function. Although the
site of this action suggested for the active principle of liver extracts is unknown,
the possibility that it may be in the liver merits consideration. For if this were
confirmed it would provide support for the view that the lack of response to paren-
teral liver extracts displayed by certain types of megaloblastic anemias is due to
hepatic dysfunction. Moreover, the action of choline in overcoming the refractori-
ness of such cases would be comprehensible in view of the recognized influence of
this substance upon the metabolism of liver cells.

The satisfactory response of cases of this type to oral liver therapy may possibly
be explained by the assumption that the oral route results in the liberation of free
folic acid from conjugates within the alimentary tract from which it is absorbed
and utilized by the bone marrow independently of hepatic function. It must be
noted, however, that Doan refers to a case of megaloblastic anemia complicated
by hepatic cirrhosis which was refractory to folic acid.

The apparent "boosting" effect of choline in those cases in which a response to
liver extracts has already occurred may conceivably be due to the action of choline
in stimulating relatively healthy liver cells to further activity which, in the pres-
ence of a residuum of the active principle, enhances the production of folic acid.

Acceptance of this theory of hepatic dysfunction as the cause for refractoriness to
parenteral liver does not, in our view, necessarily imply that the disorder is caused
by an inadequate intake of choline. In the presence of established disease, the cho-
line requirements of the liver may well rise considerably above the normal. The
therapeutic effect of the administration of choline does not therefore justify the
assumption that nutritional deficiency is necessarily concerned in etiology.
Although plausible, this theory of hepatic dysfunction as a cause of refractory megaloblastic anemia is obviously based upon very slender evidence, and the objections to it are manifest. It is well known that the functional reserve of the liver is considerable and that adequate function is compatible with extensive pathological changes. In none of our cases were there manifestations of hepatic dysfunction other than slight icterus and urobilinogenuria, although it must be admitted that liver biopsies and comprehensive liver function tests were not performed in cases 9 and 10. Moreover, many cases of pernicious anemia show marked jaundice, and presumably in long-standing untreated cases fatty and other changes are frequently present in the liver, yet lack of response to parenteral liver therapy is exceedingly rare.

Apart from its influence upon the liver, other effects of choline must be considered in seeking an explanation of its erythropoietic activity. Thus, it is possible that this is due to the influence of choline upon the metabolic activities of other tissues such as the bone marrow. Alternatively, the erythropoietic action of choline may result not from its lipotropic or other influence upon metabolism but simply from its vasodilator effect. Although choline has been shown to depress erythropoiesis in normal animals (Davis), it is conceivable that it may exert a reverse effect under the abnormal conditions obtaining in megaloblastic anemias.

A point already referred to, but one deserving further emphasis, is that the demonstration of an erythropoietic response to choline does not necessarily imply that the patient was suffering from a nutritional choline deficiency. The established pharmacological effects of choline and related substances are almost certainly the result of its action, in relatively high concentration, upon susceptible cells and not due to the restoration of a normal physiological level. This question is of some practical importance, because if the response to choline is due to the correction of a deficiency, it follows that refractoriness to parenteral liver may be due to faulty nutrition. This seems improbable, however, since our patients in whom choline was effective presented no unusual subjective or objective evidence of nutritional deficiency, while the patients who were believed to be in an unsatisfactory nutritional state showed no response to choline. Although this reasoning is not conclusive, it does suggest that if choline deficiency plays a significant part in the etiology of the type of case under discussion, it is probably conditioned by an intrinsic defect, rather than the result of inadequate intake.

**Dosage and Route of Administration of Choline**

Our data are too scanty to justify dogmatism regarding the optimal dosage and method of administration of choline chloride. Nevertheless, consideration of case 9 suggests that while choline given by mouth is not without some erythropoietic effect, it is probably more efficacious when administered intravenously, despite a considerable reduction in dosage. The reason for this is difficult to explain, since numerous animal experiments have shown that choline by mouth exerts an effective lipotropic action. Presumably the intravenous injection, even of relatively small doses, results in a higher, although more transient, blood concentration of choline than the administration of larger doses by mouth. This perhaps suggests that the
The erythropoietic effect of choline depends upon a positive action rather than upon the rectification of a deficiency. On the other hand the possibility must be considered that interference with absorption of choline from the alimentary tract may be responsible for the greater efficacy of its intravenous administration.

The difficulties of high dosage by the intravenous route have already been discussed. Daily intravenous injections of 1 gram of choline chloride, however, were usually without unpleasant side effects, and since this dosage was effective in several of our cases, as well as in that of Moosnick et al., it seems probable that, in cases likely to respond to choline, this dose may be adequate. The possibility must be admitted that a higher dosage may be desirable to ensure the optimal erythropoietic effect, although it should be noted that some of our patients who did not respond to choline received it in doses of 10 grams daily, both orally and intravenously.

THE CLINICAL VALUE OF CHOLINE AS A HEMATINIC

Our results do not suggest that choline will be of much practical value in the treatment of megaloblastic anemias, because only a small proportion of cases refractory to parenteral liver therapy are likely to respond to choline. On the other hand, there are good reasons for believing that the great majority, if not all, of such cases can be treated effectively with oral liver preparations. It is possible, however, that choline may be of value in exceptional cases complicated by severe hepatic disease, such as that of Moosnick et al., but this point cannot be decided until more information is available concerning the effect of oral liver or folic acid therapy in this type of case.

Despite the limited applications of choline in therapeutics, the apparent erythropoietic effect of this substance seems to be of sufficient academic interest to warrant further study, which might well be extended to embrace other substances having similar pharmacological actions.

SUMMARY AND CONCLUSIONS

1. The effect of the administration of choline chloride has been observed in 10 cases of megaloblastic anemias of various types.
2. Choline was without effect in a case of untreated Addisonian pernicious anemia which subsequently responded to parenteral liver therapy.
3. Choline was also without effect in a case of nutritional megaloblastic anemia, in a case of megaloblastic anemia of pregnancy, and in two cases of megaloblastic anemia associated with the sprue syndrome. All these cases had proved refractory to injections of potent liver extract before the choline was given, and all responded to subsequent oral liver or folic acid therapy.
4. A significant erythropoietic response to choline occurred in two cases resembling Addisonian pernicious anemia which were refractory to parenteral liver extracts.

Secondary responses followed the administration of choline in two other cases of Addisonian pernicious anemia and in a case of megaloblastic anemia of pregnancy, all of which had already responded to injections of liver extract.
The significance of these observations is discussed. It is concluded that choline possesses no direct erythropoietic activity, but that under certain circumstances it may potentiate the effect of liver extracts.

It is suggested that refractory megaloblastic anemias may be divided into two groups. In one, represented by well known syndromes associated with defective absorption or pregnancy, the lack of response to parenteral liver extracts is not corrected by choline. In the other, represented by two cases simulating Addisonian pernicious anemia, choline is effective in overcoming, partially or completely, the refractoriness to parenteral liver therapy. Consideration is given to the view that the refractoriness of this group results from hepatic dysfunction.

6. The most satisfactory method of administering choline probably consists of intravenous injections in daily doses of 1 gram. Larger doses given intravenously are frequently accompanied by unpleasant side effects, while oral administration appears to be relatively less effective.

7. It seems unlikely that choline will be of practical value in the treatment of refractory megaloblastic anemias, for which oral liver preparations provide the most certain and effective treatment. It is possible, however, that choline may be of use in cases complicated by severe hepatic disease.

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REFERENCES

8. Davis, J. E.: Hyperchromic anaemia produced by choline or acetylcholine and the induced remission of both by folic acid or liver injection, the probable mechanism of action of liver and folic acid in the treatment of anaemia. Am. J. Physiol., 147: 404, 1946.
THE ERYTHROPOIETIC ACTIVITY OF CHOLINE CHLORIDE IN MEGALOBLASTIC ANEMIAS

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