# $B_{12}$ INHALATION THERAPY IN PERNICIOUS ANEMIA

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DETROIT

Of the previously suggested routes for B-12 administration in the treatment of pernicious anemia, parenteral injection alone has thus far proved satisfactory. Oral [1,2] as well as sublingual administration have resulted in unsatisfactory clinical and hematologic responses, except when given in massive doses or in conjunction with intrinsic factor.[3]

The success of parenteral injection is predicated upon the ready access of the injected material to capillaries and lymphatics. One of the largest capillary beds in the body which contacts the external environment is that of the pulmonary circulation. Inhalation therapy seemed to be a logical route for attempted administration of B-12. Reports of the effectiveness of aerosol therapy in other fields such as the antibiotics, hormones and vasoconstrictors lend support to the validity of this concept.[4]

B-12 inhalation therapy has resulted in adequate clinical and hematologic responses in three patients, two presenting severe and one mild manifestations of pernicious anemia.

## Materials and Methods

Crystalline B-12 in physiological saline solution without a preservative was utilized in the first two cases. The material was administered initially by means of pressure tank and a vaponephrin nebulizer and contained 15 mcg. of the vitamin per 1.0 ml. The dosage was that usually employed in parenteral administration of this agent. In one patient after nine days of

administration utilizing the pressure tank, self-administration was effected by means of a hand atomizer.

In the third case, 1000 mcg. of crystalline B-12 in 1/10 cc. by volume of lactose powder was utilized for administration as a dust. This was an attempt to further simplify self-administration. The B-12 lactose powder was administered by means of a dust inhaler (aerohalor-Abbott). The patient was directed to take ten deep inhalations per administration at times corresponding to usual parenteral dosage schedules.

Later experience with this method has shown that larger amounts of active material are deposited in the lungs if a forced expiration immediately precedes a slow, deep inspiration. Immediately following this inspiration the breath should be held for a few moments, then exhaled through the nose.[5]

*Case 1.* The first case, F. Z., HFH Case No. 674802, was that of a white male, age 56. He was first seen April 14, 1952,

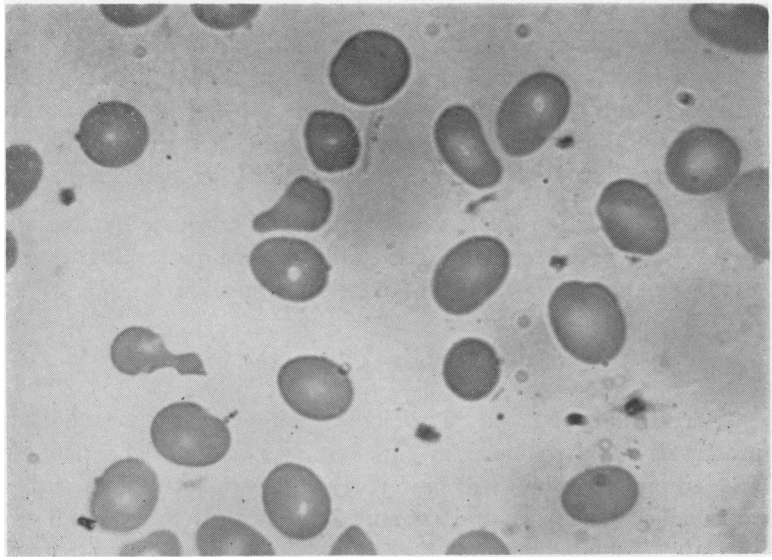

FIGURE 3.—CASE 1. Megalocytes and poikilocytes in the peripheral blood prior to treatment. X1200.

complaining of weakness, anorexia and exertional dyspnea of 8 weeks' duration. For several weeks he had noted numbness in the lower extremities. The past history was non-contributory.

Physical examination revealed a well-developed, well-nourished male with a lemon yellow skin color. There was moderate atrophy of the tongue and marked pallor of the mucous membranes. Neurological examination was normal except for mild hypesthesia of the lower extremities.

Pertinent laboratory data on admission: a gastrointestinal survey, including liver function tests and other biochemical blood determinations, was normal excepting a histamine-fast achlorhydria; Hb. 5.9 grams %; RBC 1.36 million per cu. mm.; Ht. 17.1%; MCV 125.7 cu. micra; MCH 43.3 millimicrograms; MCHC 34.5%; reticulocytes 2.2%; WBC 6,300/cu. mm.

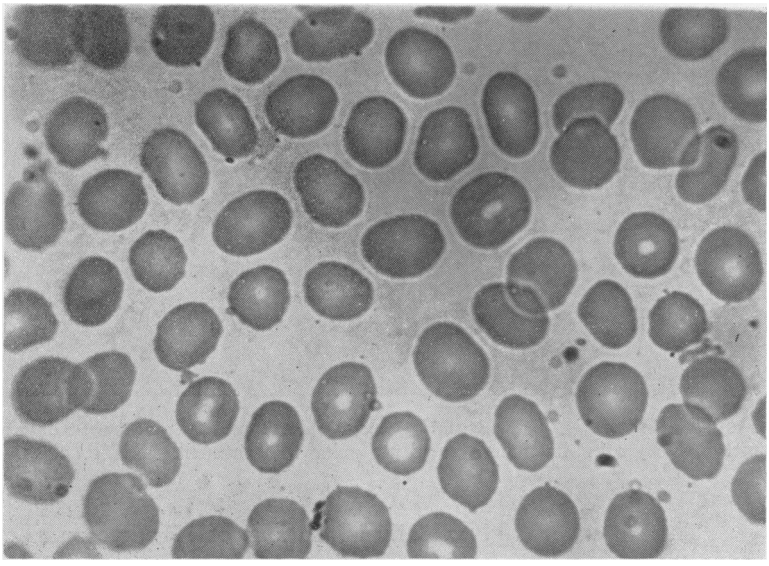

FIGURE 4.—CASE 1. Normocytes with only an occasional macrocyte in the peripheral blood after crystalline B-12 aerosol treatment. X1200.

The blood smear (Fig. 3) revealed a macrocytic erythrocytic picture characterized by the presence of megalocytes, poikilocytosis, anisocytosis and polychromasia. The neutrophilic leukocytes presented hypersegmentation of their nuclei.

The sternal aspirate revealed paradoxical hyperplasia and the presence of 44.2% megaloblasts and 12% giant neutrophils of the P.A. type indicative of marked deficiency of anti-P.A. factors. (Fig. 1, Table 1)

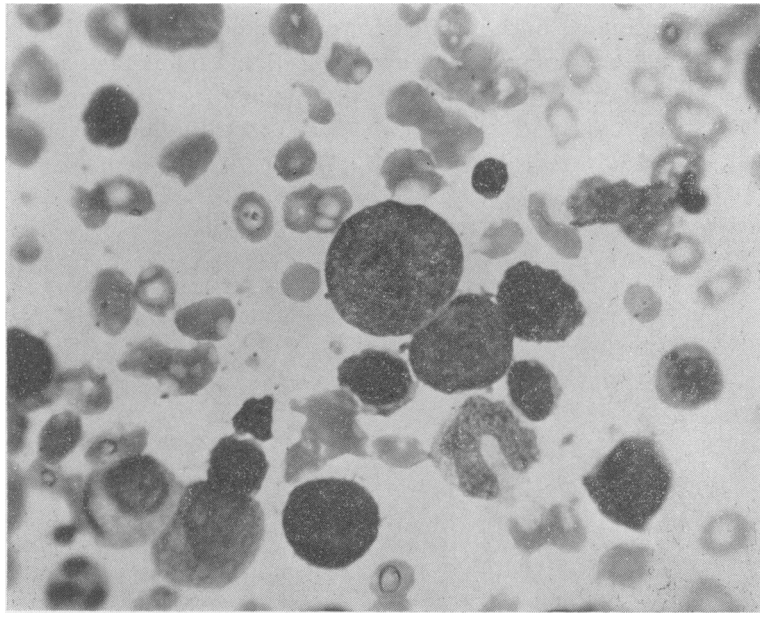

FIGURE 1.—CASE 1. Representative megaloblasts and a giant neutrophilic metamyelocyte in the marrow aspirate prior to treatment. X1200.

The patient was given daily inhalations of 15 micrograms of crystalline B-12 in 1 cc. of physiological saline. No other antianemic preparations were given.

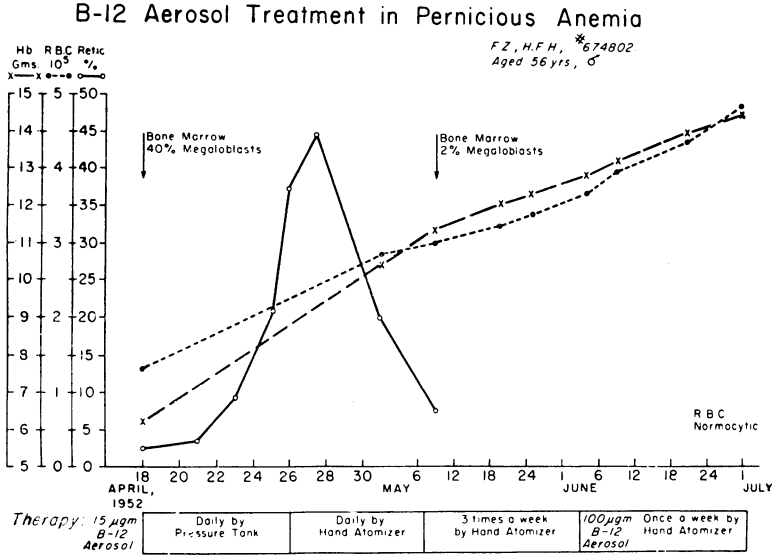


CHART 1. Hematologic response to crystalline B-12 aerosol treatment in pernicious anemia, Case 1.

The hematologic response is shown in Chart 1. On the 7th day of therapy the reticulocyte response was 21% and on the 8th day of therapy 44.2%. The patient continued on daily self-administered inhalations of B-12 at home without symptoms or signs of pulmonary irritation. A continued rise in hemoglobin and erythrocyte count has been noted on return visits to the out-patient department (Chart 1). By the 20th day the erythrocyte count had more than doubled with an increase of over 500,000 erythrocytes per week.

Examination of the marrow aspirate (Table 1) revealed a drop in megaloblasts to only 2.1% and of P.A. type neutrophils to 4.4% (Fig. 2). At this time the patient presented marked symptomatic improvement. Therapy was continued by inhalation of 15 micrograms three times a week for another month followed by inhalation of 100 micrograms in 1 ml. saline once a week by

hand atomizer. By July 1st, the erythrocyte count reached 4.68 million/cu. mm. and the Hb. 14.5 grams %.

TABLE I

DIFFERENTIAL COUNTS (1000 CELLS) OF STERNAL MARROW ASPIRATES OF PERNICIOUS ANEMIA PATIENTS BEFORE AND AFTER TREATMENT WITH CRYSTALLINE B-12 AEROSOL AND B-12-LACTOSE DUST INHALATION

| | *Before* | *After** | *Before* | *After** | *Before* | *After* |
|---|---|---|---|---|---|---|
| Myeloid:arythroid ratio | 1.1:1 | 2.4:1 | 5:1 | 5.5:1 | 0.9:1 | 5.6:1 |
| Reticulum cells (occ.) | 0.4 | 0.5 | 1.0 | | 0.6 | 0.4 |
| Myeloblasts (1.) | 0.2 | 0.4 | 0.4 | | 0.1 | 0.4 |
| Progranulocytes (2.) | 1.4 | 2.8 | 4.2 | 0.6 | 1.3 | 3.7 |
| Neutrophilic myelocytes early (4.) | 4.2 | 5.4 | 4.0 | 2.8 | 4.4 | 5.3 |
| Neutrophilic myelocytes late (13.) | 5.6 | 5.4 | 5.4 | 3.8 | 5.2 | 10.0 |
| Neutrophilic metamyelocytes (15.) | 3.2 | 7.0 | 5.4 | 4.6 | 6.6 | 8.3 |
| Band cells (16.) | 7.2 | 10.6 | 10.4 | 5.0 | 6.7 | 13.9 |
| Neutrophils segmented (15.) | 12.6 | 26.0 | 29.2 | 35.8 | 5.9 | 24.1 |
| Immature neutrophils, P.A. type (0) | 9.0 | 2.4 | 3.4 | 0.6 | 5.6 | 2.3 |
| Neutrophils segmented, P.A. type (0) | 3.0 | 2.0 | 2.6 | 1.2 | 1.3 | 0.1 |
| Eosinophilic myelocytes early | | 0.3 | | 0.2 | 0.1 | 0.2 |
| Eosinophilic myelocytes late (2.) | 0.4 | 0.8 | 0.8 | 0.2 | 0.4 | 1.6 |
| Eosinophilic metamyelocytes | 0.4 | 0.4 | 0.6 | | 0.4 | 0.6 |
| Eosinophils (1.) | 1.0 | 0.5 | 2.8 | 0.8 | 0.2 | 1.0 |
| Basophils, immature (occ.) | | 0.2 | 0.4 | | | 0.2 |
| Basophils (0.5) | | | 0.4 | | 0.5 | |
| Polymorphocytes (occ.) | | 0.1 | | | 0.3 | 0.6 |
| Lymphocytes (10.) | 5.2 | 6.5 | 11.2 | 31.4 | 7.2 | 10.6 |
| Leukocytoid lymphocytes (0) | 0.2 | | 0.2 | | | |
| Monocytes (1.) | | 1.7 | | 7.2 | 0.1 | 0.9 |
| Proplasmacyte (occ.) | | | | | | 0.1 |
| Plasmacyte (1.) | | 0.2 | 1.8 | 0.2 | 0.5 | 0.3 |
| Pronormoblasts (0.5) | | 0.7 | 0.8 | 0.2 | 1.3 | |
| Basophilic normoblasts (2.) | | 1.8 | 1.4 | 0.6 | 2.7 | 0.8 |
| Polychromic normoblasts (12.) | | 20.9 | 6.2 | 4.0 | 5.5 | 7.9 |
| Orthochromic normoblasts (4.) | 0.2 | 2.1 | 4.2 | 0.4 | 2.4 | 3.7 |
| Promegaloblasts (0) | 7.2 | 0.2 | | | 2.8 | 0.1 |
| Basophilic megaloblasts (0) | 17.0 | 0.2 | 0.2 | | 21.8 | 0.1 |
| Polychromic megaloblasts (0) | 16.6 | 1.1 | 1.8 | | 12.8 | 1.5 |
| Orthochromic megaloblasts (0) | 3.4 | 0.6 | 0.4 | | 2.2 | 0.7 |
| Phagocytic histiocytes (occ.) | 0.2 | | | | | 0.1 |
| Lipid histiocytes (0) | | | | | | 0.1 |
| Fat cells (occ.) | | | Predominate | | | |
| Mitoses, white | 0.2 | 0.1 | | | 0.5 | 0.1 |
| Mitoses, red | 1.2 | 0.9 | | 0.4 | 1.4 | 0.3 |
| Cellularity | Packed | Normal | Hy-plastic | Hy-plastic | Packed | Normal |

*Three weeks after therapy.

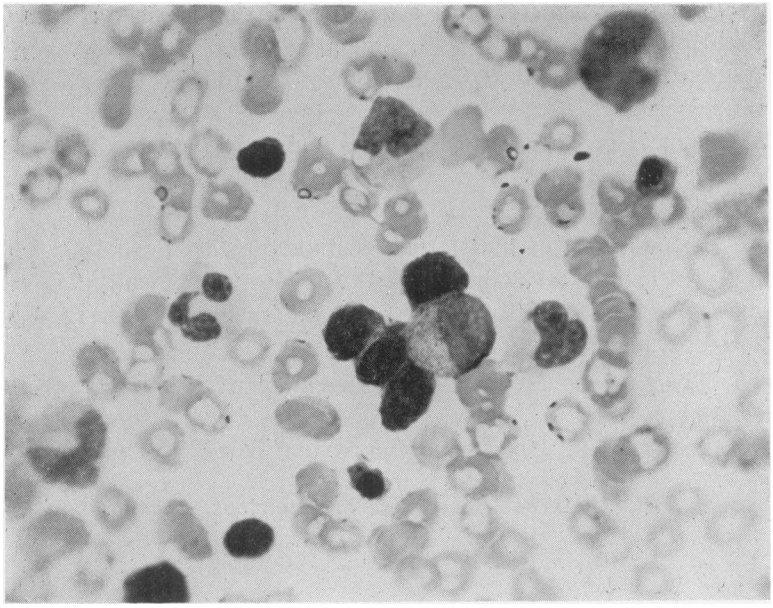

FIGURE 2.—CASE 1. Normoblasts and unaffected members of the neutrophilic series in the marrow aspirate after crystalline B-12 aerosol treatment. X1200.

*Case 2.* The second case, L. H., HFH Case No. 425149, was that of a white female, aged 71 years. She was first seen on April 20, 1952. Complaints were weakness, weight loss and paresthesias of all extremities. The past history was non-contributory.

Physical examination revealed a poorly nourished, pale, elderly female. There was mild atrophy of the tongue and pallor of the mucous membranes. Neurological examination demonstrated mild peripheral neuritis in both legs considered by the neurological consultant as consistent with the early stage of subacute combined degeneration of the cord.

A gastrointestinal survey including liver function tests and biochemical studies of the blood were normal except for the presence of histamine-fast achlorhydria.

Pertinent hematological data on admission were as follows: Hb. 12.1 grams %; RBC 3.48 million/cu. mm.; Ht. 37.8%; MCV 108.5 cu./micra; MCH 31.8 millimicrograms; MCHC 32.0%; reticulocytes 1.7%; WBC 6,200 cu./mm. Examination of the blood smear revealed a macrocytic erythrocytic picture with anisocytosis and polychromasia as additional features. An occasional neutrophilic leukocyte presented nuclear hypersegmentation.

Examination of the sternal aspirate revealed hypoplasia and the presence of 2.4% megaloblasts and 6% giant neutrophils of the P.A. type indicative of definite but moderate deficiency of anti-P.A. factors.

The patient was given daily inhalations of 15 micrograms of crystalline B-12 for 6 days, then, 3 times a week for 2 weeks. The hematologic response is shown in Chart 2. Three weeks

B-12 Aerosol Treatment in Pernicious Anemia.

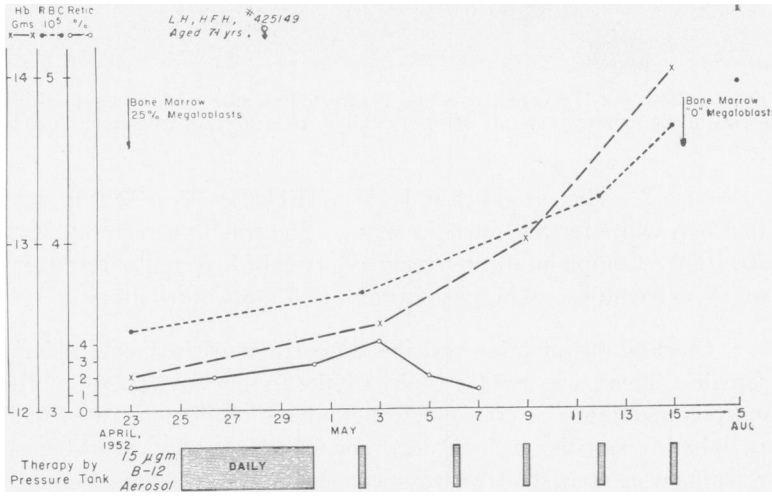


CHART 2. Hematologic response to crystalline B-12 aerosol treatment in pernicious anemia, Case 2.

after the institution of aerosol therapy the red blood cell count increased from 3.48 to 4.4 million/cu. mm. and the Hb. rose from

12.1 to 14.2 grams %. There was symptomatic improvement of the peripheral neuritis and a weight gain of 10 lb.

*Case 3.* The third case, S. G., HFH Case No. 686563, was that of a white female, aged 51 years. She was first seen August 4, 1952, complaining of weakness and sore tongue, as well as numbness and tingling of all extremities. The past history was non-contributory.

Physical examination revealed a pale, slightly obese female. A distinct yellow tint to the skin and sclerae was evident. The tongue presented a mild degree of atrophy of the lateral margins and a reddened tip. Neurological examination showed moderate hyperesthesia of the extremities.

Pertinent laboratory data on admission were as follows: a gastrointestinal survey, including liver function tests and other biochemical blood determinations, revealed cholelithiasis and a histamine-fast achlorhydria. Hb. 7.5 grams %; RBC 1.64 million/cu. mm.; Ht. 23.6%; MCV 143.8 cu. micra; MCH 45.7 millimicrograms; MCHC 31.7%; reticulocytes 1.4%; thrombocytes 100,000/cu. mm.; WBC 5,200/cu. mm. Examination of the blood smear revealed a macrocytic erythrocytic picture characterized by the presence of megalocytes, poikilocytosis, anisocytosis, polychromasia, basophilic stippling and an occasional normoblast. The neutrophilic leukocytes presented hypersegmentation of their nuclei and a slight "shift to the left."

Examination of the sternal aspirate revealed paradoxical hyperplasia and the presence of 39.6% megaloblasts and 6.9% giant neutrophils of the P.A. type indicative of marked deficiency of anti-P.A. factors. (Table 1)

The patient was given daily inhalations of crystalline B-12 in lactose powder. Ten deep inspirations per day were self-administered for eight days. No other anti-anemic preparations were given. The hematologic response is shown in Chart 3. On the 8th day of therapy the reticulocyte response was 22.0%. Study

of Chart 3 reveals a persistence of reticulocytosis above 10% for over 15 days. In the first three weeks of therapy the erythrocyte count increased from 1.64 to 2.96 million/cu. mm. or an average of over 400,000 erythrocytes per cu. mm. per week. The Hb. rose from 7.5 to 10.4 grams %. The glossitis and hyperesthesias disappeared and color and strength improved.

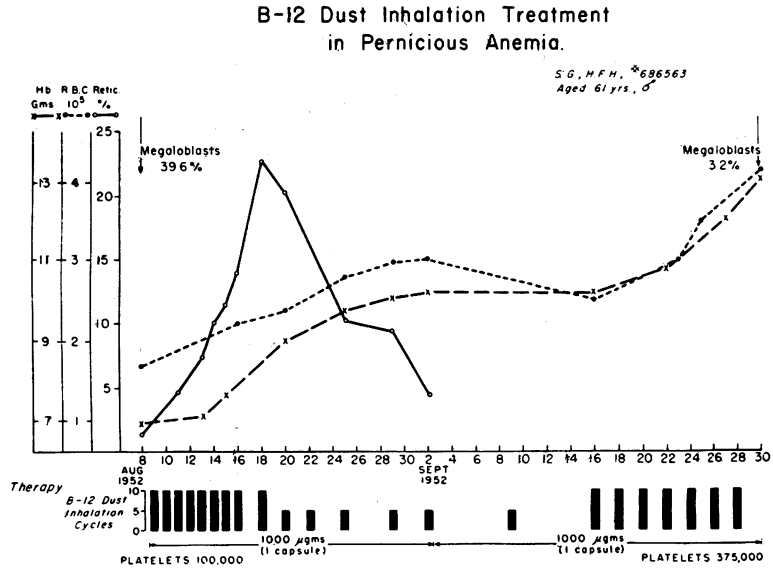


CHART 3. Hematologic response to crystalline B-12-lactose dust inhalation treatment in pernicious anemia, Case 3.

The patient was discharged to her home after 8 days of hospitalization with instructions to take 5 deep inspirations of B-12 three times a week for the first week and twice a week for the second week. In the succeeding three week period she took only 5 inspirations of B-12 lactose dust on one occasion. On this reduced dosage schedule the erythrocyte count dropped from 2.96 million/cu. mm. to 2.52 million/cu. mm. Upon reinstitution of 10 inspirations of B-12 lactose dust three times a week the patient again demonstrated an increase in her erythrocyte count at the rate of 500,000 cells/cu. mm. per week (Chart 3). Exami-

nation of the marrow aspirate (Table 1) at the end of this period revealed a drop in megaloblasts to 2.4%. The thrombocytes had risen from the original level of 100,000/cu. mm. to 375,000/cu. mm.

## Discussion

The suitability of B-12 inhalation therapy requires some amplification. Few drugs now being used in inhalation therapy meet the exacting requirements of lack of toxicity and need for closely controlled dosage. Inhalation of crystalline B-12 in physiological saline or lactose powder has, to date, produced no objective or subjective evidence of toxicity or sensitivity as the pulmonary site.

There is no question but that B-12 inhalation therapy involves additionally some degree of oral therapy including sublingual and gastrointestinal routes. However, satisfactory responses to inhalation of the vitamin were obtained at dosage levels far below those needed for effective oral therapy.[3,6]

To further evaluate the efficacy of pulmonary absorption of the inhaled B-12 over and above the evidence afforded by the clinical and hematological response previously described, urinary excretion studies were performed. These latter consisted of measurement in the urine of B-12-like growth stimulating substance, using the test organism, *Lactobacillus leichmanii*. Upon oral administration of B-12 at dosages as high as 10,000 mcgm. B-12 activity in urine has been shown to be less than 0.05 millimicrograms/ml.[2] after inhalation of only 200 mcgm. of B-12 in saline, B-12 activity in the urine reached the level of 0.26 millimicrograms/ml. and following inhalation of approximately 500 mcgm. of B-12 in a lactose powder, B-12 activity in the urine rose to 0.134 millimicrograms/ml. Direct pulmonary instillation of 100 micrograms of crystalline vitamin B-12 by means of the bronchoscope, resulted in a urinary excretion (9.76 millimicrograms/ml.) pattern equivalent to the intramuscular injection of 60-80 mcgm.

of B-12. These results indicate a definite increase in urinary B-12-like growth substance in the urine of those subjects receiving B-12 inhalation administration.

Intranasal B-12 drops and dusts, as routes of administration are being investigated.

Although these results are uniformly encouraging, they, of course, constitute only preliminary observations as to the efficiency of inhalation crystalline B-12 treatment of pernicious anemia. Additional investigations will concern the problem of long term maintenance of the pernicious anemia patient with these newer methods of specific anti-pernicious anemia therapy.

## SUMMARY

Inhalation therapy with crystalline B-12 in physiological saline and lactose dust has resulted in adequate clinical and hematologic responses in three patients, two presenting severe and one mild grades of pernicious anemia. Detectable amounts of B-12 activity in the urine were found after pulmonary administration of this substance.

## ACKNOWLEDGMENT

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## DISCUSSION

DR. CYRUS C. STURGIS (Ann Arbor, Michigan): I should like to rise to say it is new and very interesting information. There is no question that this is effective. I am surprised and interested to learn that it is.

I should like to ask two questions. One concerns whether there is any pulmonary irritation from inhalation and, secondly, what is the longest you have carried a patient on a maintenance dosage with this mode of administration.

DR. MONTO (Closing): All of the patients whom I have presented to you today did not benefit from our modified, controlled breathing which we have proven to our satisfaction greatly increases the efficiency of inhalation. Patients early in the investigation inhaled and exhaled the material without holding the breath at the end of inspiration, thus allowing for settlement. We have had patients receiving pulmonary inhalation of various forms of B-12 preparations for about eight months. There was no evidence of irritation at the time of inhalation. I personally examined the patients' chests periodically, and I was not able to determine abnormal signs in the lungs. In addition to the clinical examination, we obtained periodic roentgenograms of the chest and they showed no change.

As to the question of maintenance, we have had fifteen patients now on so-called maintenance therapy for about seven months. To date, we have had no relapses. We are at a loss as to the establishment of exact dosage schedules for the reason that many of these patients, as you might judge, demontrate varying degrees of adaptability to this modality of therapy.