OBSERVATIONS ON THE EFFECT OF AN ANIMAL PROTEIN FACTOR CONCENTRATE ON PERSONS WITH THE MACROCYTIC ANEMIA OF PERNICIOUS ANEMIA, OF NUTRITIONAL MACROCYTIC ANEMIA AND OF SPRUE, AND ON PERSONS WITH NUTRITIONAL GLOSSITIS

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For the past fifteen years, investigators have been working intensively on an illusive vitamin, or a complex of closely related factors, found in association with proteins of animal origin. For a number of years there has been evidence that soya bean meal was not adequate as the only source of protein in poultry feeds. The hatchability of eggs produced by hens fed these diets was low; whereas this defect could be remedied by feeding meat scraps.

Later it was found that a supplement of dried cow manure was effective in increasing the egg production and the hatchability of the eggs from hens fed on such a restricted diet. Whitson, Hammond, Titus, and Bird concluded that the substance was not a protein or any known vitamin. Bird, Rubin, Whitson, and Haynes aided in the search for the hatchability factor by investigating a large number of widely used foodstuffs; and Mishler, Carrick, and Hauge found that the addition of fish solubles supplied a missing factor or factors not present in a vegetable protein ration supplemented with vitamins. Rubin, Bird, and Rothchild demonstrated the presence of a chick growth factor in hen feces.

As these studies progressed on the new factor or factors necessary for the hatchability, growth, and viability in chicks, Ross, Phillips, and Bohstedt, Cunha, Spitzer and Phillips, and Cary, Hartman, Dryden, and Likely independently showed that proper growth, reproduction, and lactation did not occur in rats fed a diet of highly purified casein as the source of their protein and that they required an unidentified factor.

More recently, Zucker, Zucker, Babcock, and Hollister fed rats a purified diet containing protein and all of the known essential vitamins. The female rats maintained on this diet reproduced normally and had normal lactation, but the young born of such females frequently died soon after weaning. Crude casein, fish solubles, or liver extract corrected the deficiency, and the new factor was tentatively named "zoopherin." The authors pointed out that it is not the same as the fat-soluble "animal protein factor" of Heuser, Norris, Lucas, and Combs and of Johnson, Carrick, and Roberts.
In another field of investigation, workers found a long-sought-for antipernicious anemia factor of liver. This substance was isolated independently by workers in the United States\textsuperscript{14} and in England.\textsuperscript{15} Ott, Rickes, and Wood\textsuperscript{16} have reported that it possesses animal protein factor activity for the chick and concluded that this vitamin, termed B\textsubscript{12}, is identical or closely related to the animal protein factor from other sources.

The chemical nature and structure of the new vitamin B\textsubscript{12} is not fully known. Its molecular weight is between 1,350 and 1,750. It is a cobalt complex and contains phosphorus. Irrespective of whether this material should prove to be identical with one or more of the various chick growth and hatchability factors, vitamin B\textsubscript{12} is of great interest in human nutrition. There are substantial differences in nutrient needs among the species, but we must suppose that man, as well as other animals, is greatly dependent on such factors in his diet for protection. The appraisal of the worth of these particular substances to human beings is being determined by observations of therapeutic responses and by biochemical examinations of the body tissues and fluids.

It has been shown recently that concentrates from a micro-organism having "animal protein factor" activity were effective in inducing blood regeneration in two patients with pernicious anemia.\textsuperscript{17}

The studies reported in this communication were devised to provide the answers to the following questions:

1. Could we confirm the findings of Stokstad and his associates that a concentrate produced by micro-organisms, which acts as a source of animal protein factor as measured by assay with chicks, would be effective in producing a positive hemopoietic response in persons with Addisonian pernicious anemia in relapse?

2. Would this same concentrate be effective in producing blood regeneration in persons with nutritional macrocytic anemia in relapse?

3. Would this concentrate produce a blood response in persons with the macrocytic anemia of tropical sprue in hemopoietic relapse?

4. Would it produce a remission of nutritional glossitis unassociated with macrocytic anemia?

For these studies we selected 5 cases of pernicious anemia, 4 cases of nutritional macrocytic anemia, and 3 cases of nutritional glossitis from the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, and 3 cases of tropical sprue from the Pabellon Especial, the ward for the study of sprue, in the General Calixto Garcia Hospital, Havana, Cuba.

An important criterion in the selection of all the patients was a painful, fiery red tongue.

The following four criteria were used in the selection of all the patients with pernicious anemia, nutritional macrocytic anemia, and tropical sprue: (1) macrocytic hyperchromic anemia; (2) red blood cell count of 2,500,000 or less; (3) color index of 1.0 or more; (4) megaloblastic arrest of the sternal bone marrow.

An additional criterion for pernicious anemia was the absence of free hydrochloric acid in the gastric contents after histamine stimulation. Additional criteria for nutritional macrocytic anemia were the presence of free hydrochloric acid in
the gastric contents and diarrhea with liquid to soft, brown stools. Additional criteria for tropical sprue were the presence of free hydrochloric acid in the gastric contents, a flat glucose tolerance curve, acid steatorrhea, and loss in body weight.

All the patients except Case 1 were ambulatory and came to the hospital daily for observation and treatment. Repeated gastric analyses were made in each case. Daily studies of the peripheral blood included red and white blood cell counts, hemoglobin determinations, and reticulocyte counts made by methods previously described. Bone marrow studies were made prior to therapy.

After the baseline determinations were completed, animal protein factor concentrate was injected in amounts ranging from a total of 5 cc. in a period of twenty-three days to 5 cc. daily for fourteen days. The following brief representative case histories illustrate the responses of one case of pernicious anemia, one case of nutritional macrocytic anemia, one case of tropical sprue, and one case of nutritional glossitis unassociated with anemia.

**Case Reports**

**Case 1.** M. D., a 62 year old white woman, was admitted to the Hillman Hospital, Birmingham, Alabama, in October, 1948, complaining of 'weak spells' and numbness and tingling of the hands, legs and feet. Because her memory was poor, she was unable to give an accurate history. She stated that her illness began rather suddenly four years previously and was characterized by weakness and epigastric distress. At that time she remained in bed for three months but could not recall having had any specific treatment. She gained enough strength slowly to become ambulatory but she continued to feel weak. In 1947, after a period of progressive weakness, she had again become bedridden. She lost her appetite, lost weight, and developed edema of the ankles and numbness and tingling of the feet, legs, and hands. She was in bed for seven months, and had numerous injections which she thought were liver extract. Following this treatment, she was able to be up for three months although the numbness of the feet, legs, and hands persisted and she felt weak. The following seven months she spent most of the time in bed. She failed to gain strength and came to the Nutrition Clinic seeking treatment. She was admitted to the Hillman Hospital, where physical examination showed a poorly developed, poorly nourished woman. The skin was pale and showed decreased elasticity. The conjunctivae were very pale and the sclerae had a slight icteric tint. The tongue showed mild glossitis. She walked with a staggering gait and watched the movement of her feet carefully. The calves were slightly tender to pressure. There was hypesthesia of the hands and forearms, hypesthesia of the fingers and hyperesthesia of the lower thighs and the legs. Touch perception in the feet was absent. There was slight blunting of position sense. The knee jerks were hyperactive. Vibratory sensation showed a great decrease at the ankles and a slight decrease at the knees and wrists. Repeated gastric analyses showed no free hydrochloric acid in the gastric juice after histamine stimulation. The blood values on admission were: red blood cells 1.73 million; white blood cells 1,900; hemoglobin 6.6 grams (41 per cent); and reticulocytes 1.6 per cent. The patient was given 5 cc. of animal protein factor concentrate intramuscularly each day for fourteen days. The reticulocytes began to rise on the sixth day of therapy and reached a peak of 16 per cent on the seventh day (fig. 1). Three weeks after therapy was initiated, the red blood cell count increased to 3.41 million; the white blood cell count increased to 8,750; the hemoglobin increased to 9.3 grams (60 per cent); and the reticulocytes decreased to 2.8 per cent. Eight weeks after therapy was discontinued, the red blood cell count was 3.88 million; the white blood cell count 7,750; the hemoglobin 11.4 grams (80 per cent); and the reticulocytes 1.0 per cent. The sixth day following the initiation of therapy, she had a definite improvement in appetite and consumed more food than she had previously. By the seventh day the signs of glossitis had subsided, and she stated that she felt stronger and began to spend time out of bed. At this time she seemed a little more alert mentally, and she began to complain more severely of pains and paresthesia of all the extremities. Objectively, no improvement in the neurologic status could be detected except that she seemed to walk a little better and this might very well be attributed to a gain in strength rather than to any real improvement in the nervous system.
Case 2. E. S., a 78 year old white man, had been under observation since 1943, at which time a diagnosis of nutritional macrocytic anemia was made. Following treatment with liver extract, he had had...
an excellent hematologic response, and the mild glossitis that was present prior to therapy disappeared. He continued to eat a very inadequate diet as he had done for several years, worked every day, and did not receive maintenance therapy. His anemia relapsed each year for the following four years and each time was accompanied by a moderately severe glossitis. Liver extract administered at the time of each relapse was followed by a good hemopoietic response and relief of the glossitis. In January, 1948, the anemia relapsed again and he had a recurrence of mild glossitis. Following the administration of 10 mg. of folic acid by mouth daily for fifty-seven days, he showed an excellent hematologic response and the glossitis gradually disappeared. He returned to his former way of life with the result that in August he had a relapse of the anemia and a recurrence of the glossitis. The glossitis disappeared and a remission of the anemia was effected following a single injection of 25 micrograms of vitamin B12. The red blood cell count rose to 3.11 million and the hemoglobin determination was 10.2 grams (67 per cent). Further vitamin B12 was not available, and five weeks later the blood values began to decrease and the glossitis recurred. He lost his appetite and complained of weakness. At this time he was given 2 cc. of animal protein factor concentrate intramuscularly daily for fourteen days. In figure 2, which shows his hemopoietic response, it can be seen that the reticulocytes reached a peak of 11.6 per cent on the ninth day of therapy. By the last day of therapy, the red blood cell count had increased from 1.87 million to 3.88 million; the white blood cell count from 7,700 to 9,100; and the hemoglobin from 10.2 grams (67 per cent) to 11.2 grams (79 per cent). The hematologic response was accompanied by a great improvement in the patient's appetite and food intake (fig. 3) and the glossitis disappeared four days after therapy was initiated.

Case 1. B. B., a 47 year old Cuban woman with tropical sprue, was treated with folic acid at the General Calixto Garcia Hospital, Havana, Cuba, in June, 1947. She had an excellent hematologic and clinical response at this time but, following her discharge from the hospital, she resumed eating a diet similar to that she had eaten for many years. It consisted chiefly of rice, commeal, bread, viandas (Cuban root vegetables), coffee, and sugar. She failed to return for follow-up studies but finally appeared at the hospital in December, 1948 when she had moderately severe glossitis and diarrhea. Her appetite was very poor and she was so weak that she could do little of her housework. Her blood values were: red blood cells 2.01 million; white blood cells 3,750; hemoglobin 10.6 grams (69 per cent); and reticulocytes 0.8 per cent.
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She was given an injection of 1 cc. of animal protein factor concentrate. Three days later she volunteered that she felt stronger and that her appetite had improved. By this time the redness of the tongue had faded considerably and the tongue was less painful. Seven days after the injection, the reticulocytes reached a peak of 8.0 per cent (fig. 4). Five days later the red blood cells increased to 1.16 million; the white blood cells to 5,250; the hemoglobin to 11.0 grams (71 per cent); the diarrhea was less severe. The following day she again complained of burning of the tongue, which showed increased redness along the border and at the tip. At this time, 1 cc. of animal protein factor concentrate was injected and this amount was given every other day until a total of 4 cc. were given. The glossitis began to subside three days after therapy was initiated. The reticulocytes reached a peak of 9.2 per cent seven days after the initial injection of animal protein factor concentrate, and by this time the diarrhea and the glossitis had disappeared.

Five days later the red blood cell count was 3.00 million; the white blood cell count 7,000; the hemoglobin 11.1 grams (78 per cent); and the reticulocytes 1.2 per cent.

Case 4. A. R., a 68 year old white woman, came to the Nutrition Clinic, Birmingham, Alabama, in April, 1948, complaining of severe soreness of the tongue which had persisted for over a year and varied in severity from time to time. It was so sore at times that she had difficulty in eating food of any kind, particularly fruit and "acid foods." She had severe general stomatitis and glossitis involving all the mucous surfaces of the oral cavity, including the gums. The blood values were: red blood cells 4.56 million; hemoglobin 12.1 grams (78 per cent). Repeated gastric analyses showed no free hydrochloric acid in the gastric juice after histamine stimulation. The patient came to the Clinic frequently for observation during the next six months and throughout this time the blood values and the glossitis remained about the same. She was then given 1 cc. of animal protein factor concentrate intramuscularly daily for three days, and she came to the Clinic daily for observation and blood examinations. Each injection was followed by local pain which lasted for about one hour. Seventy-two hours after the first injection, there was some decrease in the soreness and burning of the mouth and tongue and they were considerably less red. The
injections of animal protein factor concentrate were discontinued for four days, during which time no further improvement in the glossitis and stomatitis occurred. Then the injections were resumed in the same amounts for four days. After the second, third, and fourth doses, the patient complained of pain at the site of the injection which persisted for twenty-four hours. Examination showed areas of about 10 cm. in diameter which were red, tender, and slightly swollen. The pain and tenderness in these areas increased and the injections were discontinued at the end of four days. By this time the glossitis and stomatitis had disappeared. A subsequent intradermal test with a 1:10 solution of the concentrate gave a strongly positive reaction which developed rapidly within the first hour. At the end of twenty-four hours an area of swelling and redness with a central area of induration and tenderness of about 10 cm. in diameter remained. After forty-eight hours there was a residual area of induration and swelling at the site of the skin test.

DISCUSSION

Since the isolation of vitamin B₁₂ about a year ago, its function has been shown to be interwoven with many chemical substances. Yet the scientific story about it really begins with the findings of Minot and Murphy, which led to the inevitable conclusion that there was an active factor existing in liver and that this factor was a specific therapeutic agent against pernicious anemia. After the isolation of vitamin B₁₂, it was found that this antianemic substance for persons had animal protein factor activity as tested on chicks. A number of micro-organisms are capable of synthesizing vitamin B₁₂ and probably related chemical substances. For some time to come there will be much study and speculation on the chemical identities of these various 'animal protein factor concentrates.' At the present time the limited amount of clinical, biologic, and chemical evidence available in studying animal protein factor might suggest that this substance is identical with vitamin B₁₂. Yet a more complete evaluation is needed, and it likely will prove that once again we are dealing with a complex of chemical compounds.

SUMMARY AND CONCLUSIONS

The intramuscular injection of animal protein factor concentrate to 5 cases of pernicious anemia in relapse, 4 cases of nutritional macrocytic anemia in relapse, and 3 cases of tropical sprue in relapse was followed by a positive hematologic response in each case as is illustrated in figures 1, 2, and 4, respectively. The parenteral administration of this material to 3 patients with nutritional glossitis unassociated with anemia was followed by the disappearance of the redness and soreness of the tongue.

REFERENCES

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