Therapeutic Effect of Aureomycin in Pernicious Anemia.* (18078)

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One of the earliest theories of the pathogenesis of Addisonian pernicious anemia was that of Hunter(1). He postulated as a primary factor the presence of a toxin derived chiefly from the flora of the gastrointestinal tract and occurring in individuals constitutionally predisposed. After the demonstration of the beneficial effects of liver orally(2), liver extract parenterally(3), and the publication of Castle's work on the extrinsic and intrinsic factors(4), the maturation arrest hypothesis was accepted almost universally. Dock(5) challenged this point of view and reviewed the evidence for a hemolytic pathogenetic mechanism. Dobriner and Barker(6) showed that the bilirubin and porphyrin changes of pernicious anemia responding to liver were identical with those of hemolytic anemia after splenectomy, thus reviving the hemolytic theory of pernicious anemia. Whether or not the bacterial flora in the gastrointestinal tract is in any way implicated in the pathogenesis of pernicious anemia might be indicated by the effect of powerful antibiotics given orally to these cases.

It was reported by Stokstad and Jukes(6a) that aureomycin would produce a growth response in chicks on diets deficient in vitamin B_{12}. They suggested that aureomycin might suppress the *E. coli* population and permit the growth of other organisms which synthesize more vitamin B_{12}.

Materials and methods. We have used aureomycin† in 5 cases of Addisonian pernicious anemia in relapse and in one case of nutritional macrocytic anemia. Ages ranged from 55 to 76 years. All marrows were megaloblastic. Complete blood counts and hematocrits were done 3 times a week, the red blood cell counts being done in duplicate. Reticulocyte counts were performed daily. The therapy of each patient will be presented in detail below.

Results. The data are recorded in Table I. In those patients who were transfused before onset of therapy, the initial red blood cell counts recorded are those after transfusion. The first patient (Case No. 1) with Addisonian pernicious anemia was treated with 2 g of aureomycin and 2 g of streptomycin orally each day for 32 days (Fig. 1). This resulted in a delayed reticulocyte peak of 9.6% with a slow concomitant rise in blood values. Bacteriological studies of the stools revealed a gradual decrease in all bacterial growth until the end of the second week of therapy when unidentified yeasts were found in great numbers with almost complete absence of other organisms. The yeasts persisted until the 25th day when they were replaced by almost a pure culture of *Pseudomonas aerogenosa.* In this experiment a good clinical response and a definite, though submaximal, hematological response was elicited in a patient with Addisonian pernicious anemia by the use of oral antibiotics alone. The traces of vitamin

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1. Hunter, W., *Severest Anaemias*, 1909, London, Macmillan & Co.

2. Minot, G. P., and Murphy, W. P., *J. A. M. A.*, 1926, v87, 470.

3. Cohn, E. J., *J. Biol. Chem.*, 1927, v74, 49.

4. Castle, W. B., Townsend, W. C., and Heath, C. W., *Am. J. Med. Sci.*, 1930, v180, 305.

5. Dock, W., *Med. Papers Dedicated to Dr. Henry A. Christian*, 1936, 545, Baltimore, Williams & Wilkins.

6. Dobriner, K., and Barker, W. H., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 864.

6a. Stokstad, E. L. R., and Jukes, T. H., Paper presented at American Chemical Society Meetings, Philadelphia, March, 1950.

† The aureomycin used in each of the cases except the first was supplied to us by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y. Their microbiological assay of this lot revealed the presence of 0.17 μg of vitamin B_{12} per gram of aureomycin.

TABLE I.

Case No.	1		2		3		4		5	
Day of R_x	Retic. %	RBC mil./cmm.	Retic. %	RBC mil./cmm.	Retic. %	RBC mil./cmm.	Retic. %	RBC mil./cmm.	Retic. %	RBC mil./cmm.
0	0.4	2.10	1.8	2.71	1.7	1.54	1.5	1.60	1.0	1.68
4	0.2		3.9	2.68	1.5	1.37	6.5	1.70	3.3	1.71
8	0.8	2.89	4.2	2.72	0.9	1.33	1.2	1.73	6.9	2.01
12	1.4		2.8	2.91	1.4	1.24	1.7	1.69	4.7	1.83
16	1.6	2.66	1.9	2.90	2.1	1.18	3.3	1.82	2.3	2.30
20	5.2		2.3	2.81	3.6	1.58	3.6	1.68	1.7	2.42
24	9.6	2.82	3.6	3.01	5.8	1.29	5.2	2.10	1.3	2.47
28	6.2		4.6	3.32	12.2	1.81	4.8	2.18	2.2	2.51
32	2.8	3.21	4.6	3.09	4.8	2.29	1.2	2.56	1.2	2.66
36			3.1	3.05	3.6	2.64	3.9	2.28	1.2	3.06
40			4.3	3.32	2.0	2.83	2.4	2.40		
44					0.9	3.01	3.1	2.64		
48					1.1	3.20	2.5	3.05		

Hematological results of aureomycin given to Cases 1, 2, 3, 4 who had Addisonian pernicious anemia, and to Case 5, who had nutritional macrocytic anemia. Therapy was given orally daily as follows:

No. 1—2 g aureomycin and 2 g streptomycin 0-32nd day.
No. 2—3 g aureomycin 0-40th day.
No. 3—(a) 3 γ B_{12} 0-48th day.
(b) 3 g aureomycin 11-48th day.
No. 4—(a) 2 g aureomycin 0-28th day.
(b) Meat-free diet 0-12th day.
(c) 200 g meat daily 13-28th day.
No. 5—(a) Meat-free diet and 2 g aureomycin 0-36th day.
(b) 3 γ B_{12} 13-36th day.

B_{12} that may have contaminated the aureomycin and streptomycin used here could not possibly have been solely responsible for the therapeutic effect. It has been repeatedly demonstrated that vitamin B_{12} is ineffective orally(7-9) in pernicious anemia except in extremely large amounts or with normal gastric juice.

A second patient was given 3 g of aureomycin daily for 40 days, during which time the hematocrit rose from 33% to 43%, with a slower increase of red blood cells. There was an irregular reticulocytosis of 2-5% from the 5th day of therapy throughout the entire course. This is not the conventional response and therefore cannot be interpreted. Clinical improvement was very satisfactory and was characterized by better appetite and loss of fatiguability.

7. Spies, T. D., Suarez, R. N., Lopez, G. G., Milanes, F., Stone, R. E., Toca, R. L., Arambura, T., and Kartus, S., *J. A. M. A.*, 1949, v139, 521.

8. Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R., and Epstein, M., *New England J. Med.*, 1948, v239, 911.

9. Unpublished data of the authors.

In the first 2 cases the beneficial effects of the antibiotics resulted without the addition of vitamin B_{12} to that present in the normal hospital diet. It seemed possible that aureomycin might be active hematopoietically through its ability to augment utilization or absorption of vitamin B_{12}. In Case No. 3 (Fig. 2) therefore, vitamin B_{12} was administered orally in a dosage of 3 μg a day. During the first 10 day period this was ineffective, but, with the subsequent addition of aureomycin, there was a clinical and hematological response of a greater magnitude than in the preceding cases receiving antibiotics alone.

It was thought that the effect of extrinsic factor in the form of meat might be enhanced by aureomycin in a manner similar to that of extrinsic factor in the form of vitamin B_{12} in the previous experiment. Case No. 4 was given a diet free of meat, eggs, fish and cheese, plus 2 g of aureomycin orally each day. During the first 12 days there was no response. On the 13th day a daily dietary supplement of 200 g of lean meat was started The red blood cells began to rise slowly but steadily

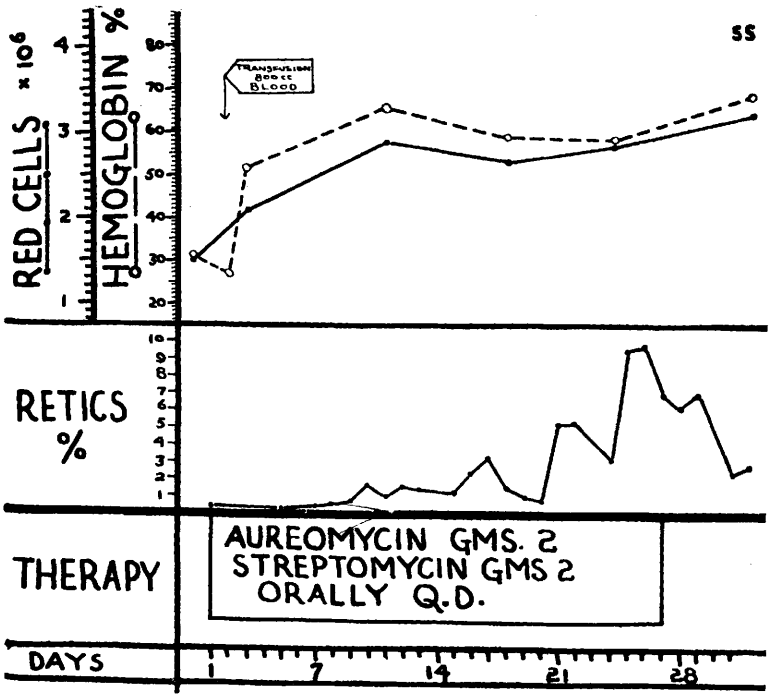

FIG. 1.
Hematological response of Case No. 1 (Addisonian pernicious anemia) to antibiotics alone.

to a value of 3.62 mil per cmm on the 61st day of the experiment. There was an irregular, non-specific type of reticulocytosis which could not be interpreted in relation to therapy. The rise in blood values was associated with an excellent clinical response and a conversion of the bone marrow to a normoblastic type. Since it is well known that a patient with Addisonian pernicious anemia will not respond to administration of meat alone, it was thought that the response here was brought about by the aureomycin.

A study similar to Case No. 4 was performed on a patient (Case No. 5) who had nutritional macrocytic anemia with free HCl in the gastric juice. This patient was given a diet free of meat, fish, eggs, and cheese, and 2 g of aureomycin orally each day. During the first 13 days on aureomycin with no extrinsic factor available to the patient there was an irregular reticulocytosis and a rise in blood elements along with some clinical improvement. The addition of oral vitamin B_{12} for a second period of 18 days seemed to cause no definite secondary response. The later successive parenteral administration of vitamin B_{12} and pteroylglutamic acid (PGA) did not produce another rise in reticulocytes or further rise in red blood cell values.

In all 5 cases the marrow was found to have become normoblastic upon completion of aureomycin therapy. Clinical response was slower than that observed in patients responding to parenteral vitamin B_{12} or liver extract, but was satisfactory. No exacerbations of neurological symptoms were observed. It is interesting that 3 of the 5 cases exhibited an irregular reticulocytosis while on aureomycin. The reason for this is obscure.

Finally, a 6th patient, with Addisonian pernicious anemia, was treated with intravenous aureomycin in order to determine whether the route of administration might be a factor. The initial red blood cell count was 1.68 mil per cmm. The patient was given 600 mg of aureomycin intravenously each day for 20 days, without any clinical or hematological response whatsoever. The re-

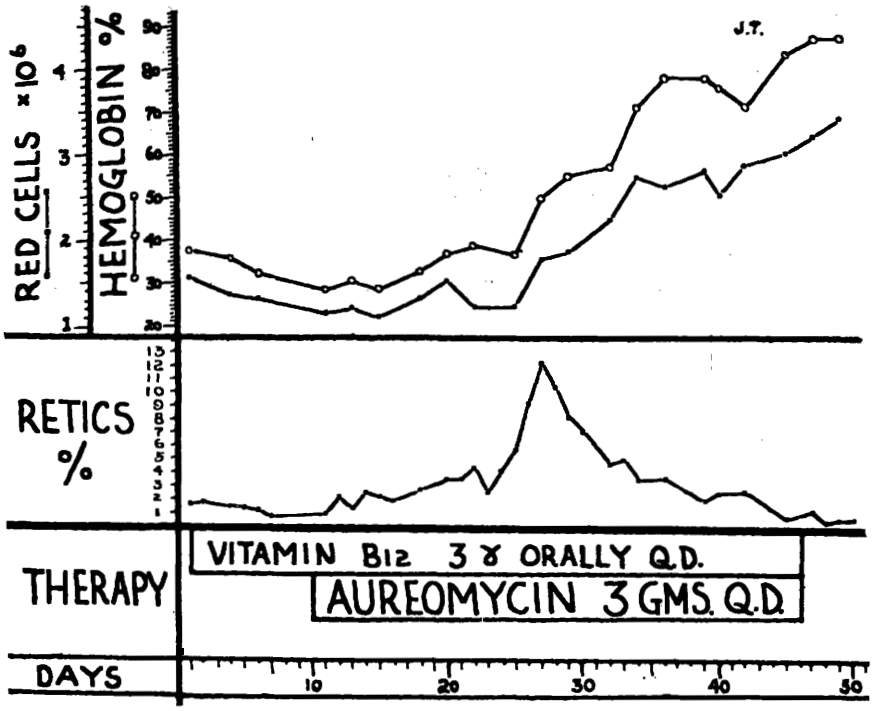

FIG. 2.
Hematological response of Case No. 3 (Addisonian pernicious anemia) to vitamin B_{12} orally only after addition of aureomycin.

ticulocytes never rose above 2.0%. The lack of response was not unexpected because it was thought that the probable site of action of aureomycin was in the intestine, and presumably intravenous administration would not give as high a concentration in the intestine as oral administration. However, further experiments are necessary before definite conclusions can be reached.

Discussion. Although potent therapeutic agents—vitamin B_{12}, PGA, and liver extract —are available for the treatment of pernicious anemia and other related megaloblastic anemias, the pathogenesis of these disorders has not been completely elucidated because the spontaneous remission is unexplained by current theories. If the development of the state of pernicious anemia depends simply upon a failure to absorb vitamin B_{12} due to achylia gastrica, who do so many people with achylia remain immune? The bacterial flora theory had many adherents before the discovery of the beneficial effects of liver. Moensch(10) studied the fecal flora in 33 cases of pernicious anemia and reviewed the pertinent literature. Their results coincided with the finding of others, that there was an unusually large number of viable organisms in the stools of these cases. Particularly numerous were *E. coli* and *Cl. welchii*. Cameron *et al.*(11) demonstrated the production of a macrocytic anemia in rats by the formation of a blind loop in the small intestine. The manner of the development of the anemia in their animals suggested to them "that the decisive event may be a change in the flora of the loop and, possibly, in the remainder of the small intestine. This may lead to the loss of an organism which synthesizes hemopoietic material, or the preponderance of an organism which uses up hemopoietic material, or the presence of an organism which produces an antagonist to hemopoiesis." The anemia

10. Moensch, M. L., Kahn, M. C., and Torrey, J. C., *J. Infect. Dis.*, 1925, v37, 161.

11. Cameron, D. G., Watson, G. M., and Witts, L. J., *Blood*, 1949, v4, 803.

in these rats responded to liver extract and PGA. Unfortunately we have not had an opportunity to study the effect of aureomycin in patients with megaloblastic anemia due to intestinal disease.

The mechanism of action of the aureomycin in our cases remains to be determined. A few of the many possibilities are: 1. The sterilization of the gastrointestinal flora by aureomycin may remove a hemolytic toxin. 2. The lowering of the bacterial population in the gastrointestinal tract may increase the amount of dietary vitamin B_{12} available for the patient. Recent work(12) has demonstrated that *E. coli* has a marked affinity for vitamin B_{12} and can remove it from solution ***in vitro***. 3. The lowering of the bacterial flora count by aureomycin may permit the growth of other microorganisms, for example, yeasts, which in turn may produce PGA or other hematopoietically active substances utilizable by the patient. In the only case (Case No. 1) in which studies of the bacterial flora were done we found a great increase in the presence of yeasts. Dearing and Heilman(13) noted a similar increase in yeasts in the stools of the majority of their patients after the use of aureomycin for short periods of time. Smith and Robinson(14) reported that large doses of streptomycin administered to mice greatly reduced the coliform organisms with a concomitant rise in ***Bacillus megatherium*** which has been reported by McGinnis and co-workers(15) to produce large quantities of vitamin B_{12}. 4. Aureomycin may have a specific hematopoietic action not dependent upon its antibiotic activity. This seems unlikely, especially in view of the lack of response to aureomycin given intravenously in our last case. All other agents effective in the treatment of pernicious anemia have been much more effective when given parenterally than when given orally.

Conclusions. Four patients with Addisonian pernicious anemia in relapse and one patient with nutritional macrocytic anemia were treated with aureomycin given orally, with definite although submaximal hematological improvement. A fifth patient with Addisonian pernicious anemia was treated with aureomycin given intravenously with no response.

We are indebted to Mrs. Helen Jakubowski for performing the blood examinations.

12. Davis, B. D., and Mingioli, E. S., *J. Bact.*, in press.

13. Dearing, W. H., and Heilman, F. R., *Proc. Mayo Clinic*, 1950, v25, 87.

14. Smith, C. G., and Robinson, H. J., *J. Bact.*, 1945, v50, 613.

15. McGinnis, James, Stephenson, E. L., Levadie, B. T., Carver, J. S., Garibaldi, J. A., Ijichi, K., Snell, W. S., and Lewis, J. C., *Abstracts Am. Chem. Soc. Meeting*, Sept., 1949, p. 42.

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