THE RESPONSE OF MEGALOBlastic MACROCYTIC ANAEMIA TO CRYSTALLINE PENICILLIN G PRELIMINARY REPORT

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During the course of work on the anaemias of Africans in Kenya we were often struck with the reticulocyte responses which followed the administration of crystalline penicillin G for reasons other than anaemia. However, as these patients had generally been given iron, "marmite," or liver, we did not consider that penicillin could be regarded as the only effective agent. We decided, therefore, to treat a case of macrocytic megaloblastic anaemia with nothing but penicillin.

In carrying out these experiments we believed that penicillin might be effective in these dyshaemopoietic anaemias, as a result of the work that had recently been done on changes in the intestinal flora that occur after antibiotic treatment.

We considered that if an abnormal intestinal flora was inhibiting the synthesis, absorption, or utilization of essential haemopoietic factors, then penicillin might be regarded as a means of dealing with this. We also had in mind the work of Cameron *et al.* (1949), in which it was suggested that in the macrocytic megaloblastic anaemias of intestinal stricture there might be changes in the flora of the intestines which might lead to (1) loss of an organism that synthesizes haemopoietic factors, (2) production of an organism that competes for haemopoietic factors, or (3) the production of antagonists. It is known that penicillin prevents the assimilation of glutamic acid by certain bacteria (Gale and Taylor, 1946), and this may affect the synthesis of pteroylglutamic acid.

There is also the possibility that penicillin might have been acting directly as a haemopoietic factor in a similar way to liver, folic acid, marmite, or B₁₂. There is the additional possibility that penicillin may be affecting the metabolism, utilization, and excretion of certain essential amino-acids, since it is known that penicillin is derived from amino-acids. In this connexion we may mention that a number of urines from our cases of anaemia show the comparatively rare "T-spot" in the distribution of amino-acids on the chromatograms prepared by Dr. C. E. Dent, of University College Hospital Medical School. "T-spot," according to Dr. Dent, is an unknown amino-acid* probably present in traces in all human urines, but which constantly occurs in large quantities in 4-5% of normal persons. When present in such quantities it tends to be associated with a characteristic pattern of the other commonly occurring amino-acids. This urinary abnormality is hereditary (Dent and Harris, unpublished work). Excessive excretion of "T-spot" may also occur in liver disease (Dent and Walshe, 1950). Until more analyses have been

done the question whether the "T-spot" distribution in these cases from Kenya is pathological or accidental must be left open. The glutamic acid present in such high concentration is probably an artifact due to breakdown of complexes during the transit of urine from Nairobi to London (Dr. Dent, personal communication).

The megaloblastic macrocytic anaemia that occurs in *Dibothriocephalus latus* infections led us to postulate that the presence of this parasite or other helminths in the intestine might in some way interfere with synthesis, utilization, or absorption of haemopoietic principles (Foy, Kondi, and Hargreaves, 1950; Foy, Kondi, Hargreaves, and Lowry, 1950).

With these possibilities in view, we treated a case of megaloblastic macrocytic anaemia with nothing but penicillin injections. The results, which are given below, indicate that the response was exactly similar to that obtained with any haemopoietically active substance. The reticulocyte response was maximal for the blood count and was followed by red cell and haemoglobin regeneration. In addition, the bone marrow, which contained both megaloblasts, and giant stab-cells before treatment, showed a complete and rapid disappearance of the former and a slower disappearance of the latter. This is exactly similar to what we have found to occur in the megaloblastic macrocytic anaemias of Macedonia and Kenya when treated with a potent liver extract, folic acid, or B₁₂. The Price-Jones curve, which had originally shown anisocytosis and macrocytosis, reverted to the normal pattern.

Case History

A Kikuyu woman reported to Dr. Brian Harris at the Kiambu Native Hospital in May, 1949, with an anaemia, having a red cell count of 2,790,000 and a haemoglobin of 65%. No facilities for more extended examinations were available at Kiambu. She was treated by Dr. Harris with 2 ml. of "neo-hepatex" for five days, recovered, and was discharged. In August, 1949, the woman again appeared at Dr. Harris's dispensary with an anaemia. No blood count or haemoglobin estimations were done. 2 ml. of neo-hepatex was again given for five days and the patient was discharged well. In January, 1950, the woman appeared once more, and on this occasion Dr. Harris put the case at our disposal. She was transferred to the Nairobi Group Hospital on January 10, 1950.

First Admission

Physical examination showed an extremely anaemic well-nourished negress of good physique, aged about 30; she had never been pregnant; she was icteric and her ankles were slightly oedematous; the spleen and liver were not enlarged; her tongue, teeth, and nails were all normal, and she had no abnormal neurological signs. Venous blood was taken without any constriction, and the laboratory findings are given fully in Table I and Fig. 1.

The Price-Jones curve is shown in Fig. 2, and harmonizes with the M.C.V. of 97 μ^3 . This picture fits in with that previously described for many of the anaemias of Kenya (Foy *et al.*, loc. cit.).

A gastric test was done after histamine, and showed the following free acid in N/10 NaOH equivalents: fasting, 2 ml.; 15 minutes, 0.5 ml.; 30 minutes, 4 ml.; 60 minutes, 4 ml.; 120 minutes, 2 ml. Like so many of our cases here, she was hypochlorhydric (see below).

Five separate examinations of the stools revealed no intestinal parasites. Her marrow contained typical Ehrlich's megaloblasts and giant stab-cells (Foy, Kondi, and Hargreaves, 1950; Foy, Kondi, Hargreaves, and Lowry, 1950).

The patient was kept under observation from January 10 to 18 with no treatment whatever. The reticulocyte response

*Since this article was prepared for publication the "T-spot" has been identified as β -aminoisobutyric acid. (Crumpler, Dent, Harris, and Westall, *Nature, Lond.*, 1951. In press.)

TABLE I.—Laboratory Findings (First Admission)

Date	Retic. (%)	R.B.C. (Mil.)	Hb (g.%)	P.C.V. (%)	M.C.V. (μ 3)	M.C.H.C. (%)	Bilirubin (mg./100 ml.)	Schumm's Test	Remarks
10/1/50 ..	5.7	1.34	4.0	13.0	103	30.6	2.8	—	Sickling negative at all pressures and after 72 hours
11/1/50 ..	2.4	1.35	4.0	13.0	97	29.2	2.6	++	Coombs test negative. W.B.C. 2,460
13/1/50 ..	3.2	—	—	—	—	—	—	—	
15/1/50 ..	4.0	—	—	—	—	—	—	—	
17/1/50 ..	6.0	—	—	—	—	—	—	—	
18/1/50 ..	3.4	—	—	—	—	—	—	—	
19/1/50 ..	6.0	—	—	—	—	—	—	—	Lung congestion. Temperature
20/1/50 ..	12.0	—	—	—	—	—	—	—	Temperature down
21/1/50 ..	11.9	—	—	—	—	—	—	—	
22/1/50 ..	16.0	—	—	—	—	—	—	—	
23/1/50 ..	25.0	—	—	—	—	—	—	—	
24/1/50 ..	26.0	—	—	—	—	—	—	—	
25/1/50 ..	37.0	—	—	—	—	—	—	—	
26/1/50 ..	33.0	2.70	6.2	—	—	—	—	—	
27/1/50 ..	24.0	—	—	—	—	—	—	—	
28/1/50 ..	19.0	—	—	—	—	—	—	—	
30/1/50 ..	8.0	—	—	—	—	—	—	—	
2/2/50 ..	4.9	3.44	7.7	32.0	94	25.0	0.8	Neg.	W.B.C. 4,300
9/2/50 ..	0.2	3.20	7.7	32.0	100	24.0	0.5	—	
21/2/50 ..	2.3	3.50	8.0	32.0	91	25.0	0.4	—	
1/3/50 ..	2.0	4.47	9.0	37.0	83	27.0	0.4	—	
13/3/50 ..	0.0	4.0	11.0	35.0	95	30.0	—	—	
23/3/50 ..	0.0	3.98	11.0	38.0	95	28.0	—	—	
24/50 ..	4.0	4.0	10.5	35.0	95	28.0	—	—	Left hospital

FIG. 1.—Results of treatment (first admission).

during this period is shown in Table I and Fig. 1. On January 18 she was put on 60 g. of marmite daily. On the 20th she developed pulmonary congestion with a high fever and was given 400,000 units of crystalline penicillin G daily for seven days to control this lung condition. During this

FIG. 2.—Price-Jones curve on January 10, 1950.

period the marmite was continued, but was reduced to 20 g. a day. From the 18th to the 25th her reticulocytes rose to 37%, the blood count to 2,700,000, and the haemoglobin to 6.2 g.%; this in spite of her pulmonary condition. The rest of the patient's haematological history is given in Table I and Fig. 1.

On February 1 *Entamoeba histolytica* cysts were found in the faeces. For these emetine treatment was instituted next day, and was continued for ten days.

The woman was discharged from hospital on April 2 with a red cell count of 4,430,000 and a haemoglobin of 8.8 g.%. The bad haemoglobin response is often characteristic of these cases here, and may be connected with low gastric acidity, low protein diet, or other unknown factors.

When the patient was discharged she was given a letter to her husband informing him that if he wanted to maintain his wife in good health he should see that she had meat at least once a day. Whether this had any effect cannot with certainty be stated, but the woman assured us at her next entry to the hospital that she had eaten meat daily.

Second Admission

On November 7 the patient again appeared at the Nairobi Group Hospital, obviously very ill and very anaemic. Her reticulocytes on entrance were 2.2%, red cell count 1,757,000, and haemoglobin 5 g.%. The complete haematological findings for her entrance examination and for the whole period of her stay in hospital are shown in Table II and Fig. 3.

A gastric test done on November 9 showed complete histamine-fast achlorhydria. Another gastric test on the 20th gave a similar result. A Price-Jones curve taken at the same time as the first blood examination is given in Fig. 4.

Four separate examinations of the stools showed no intestinal parasites. Physical examination revealed a condition similar to that on her previous admission. The liver and spleen were not enlarged, the ankles showed slight oedema, and there was the faintest suspicion of glossitis. A thorough neurological examination by Dr. Shanly showed no nervous involvement, but the patient was not very co-operative. Chest and heart were normal.

TABLE II.—Laboratory Findings (Second Admission)

Date	Retics. (%)	R.B.C. (Mil.)	Hb (g.%)	P.C.V. (%)	M.C.V. (μ^3)	M.C.H.C. (%)	Bilirubin (mg/100 ml.)	Schumm's Test	W.B.C.	Remarks
7/11/50	2.5	—	—	—	—	—	—	—	—	Feels very ill. Stools negative for parasites
8/11/50	2.2	—	—	—	—	—	—	—	—	Sickling negative
9/11/50	1.4	1.757	5.0	17.5	100	29.4	2.8	—	1,880	Headache. Stools negative
10/11/50	1.5	—	—	—	—	—	—	—	—	Nervous system shows nothing abnormal
11/11/50	1.4	—	—	—	—	—	—	—	—	Stools negative
12/11/50	3.0	—	—	—	—	—	—	—	—	Feels better
13/11/50	9.0	—	—	—	—	—	—	—	—	Much better. Stools negative
14/11/50	27.0	—	—	—	—	—	—	—	—	Stools negative
15/11/50	36.0	2.430	5.8	24.5	101	23.8	1.5	—	—	
16/11/50	29.0	—	—	—	—	—	—	—	—	
17/11/50	20.0	—	—	—	—	—	—	—	—	
18/11/50	16.0	—	—	—	—	—	—	—	—	
19/11/50	—	—	—	—	—	—	—	—	—	
20/11/50	8.0	—	—	—	—	—	—	—	—	
22/11/50	5.0	—	—	—	—	—	—	—	—	
23/11/50	3.0	3.250	8.7	31.0	98	28.0	1.0	+	3,360	
14/12/50	0.3	4.500	11.0	37.0	84	29.8	0.4	Negative	4,120	Still in hospital. Better
3/2/51	0	4.920	13.5	43.0	87	31.4	0.4	—	4,900

FIG. 3.—Haematological findings (second admission).

The patient was kept under observation until November 10, during which period the reticulocytes fluctuated between 2.2% and 1.4%. On that day she was put on 400,000 units of crystalline penicillin G daily for seven days. By November 15 her reticulocytes had reached 36%, her red cell

FIG. 4.—Price-Jones curve on November 7, 1950.

count was 2,430,000, and her haemoglobin 5.8 g. %. Results of her final examinations are shown in Table II.

A marrow puncture done on admission showed many intermediate megaloblasts and giant stab-cells. A puncture done on November 16 showed that the megaloblasts had all disappeared and there was a great reduction in the number of giant stab-cells.

Discussion

The response this patient gave to penicillin seems to us to be identical with that obtained in similar anaemias as a result of the administration of crude or refined liver, B₁₂, folic acid, or any other active haemopoietic substance. It is possible that there may have been some hidden infectious or toxic process on which the penicillin acted, thus removing a bone-marrow depressant, as sometimes happens in the case of *E. histolytica* infections when treated with emetine, or in malaria when treated with quinine. There was, however, no sign of

such infectious or toxic process in this woman, and the reticulocyte response was maximal and followed by haemoglobin and red cell regeneration such as occurs after the administration of a potent liver extract. The response of the bone marrow and the Price-Jones curve will likewise be similar to those we get after treatment with known active haemopoietic substances. From this case, we see no reason to doubt that it was the penicillin that brought about the remission.

The means by which penicillin acted on this anaemia cannot at present be stated. As we have pointed out above, it may have produced its effect by destroying an organism which was competing for some haemopoietic substance, or it may have removed a haemopoietic antagonist, or even acted directly as a haemopoietic substance. Any or all of these hypotheses might explain the response.

The question of pernicious anaemia cannot be entirely dismissed in the present case. So far as we are aware, pernicious anaemia has not been described in an adult of pure negro stock. In our opinion this case is not altogether typical of pernicious anaemia, in spite of the presence of a histamine-fast achlorhydria and the repeated relapses. The patient's response to large doses

of marmite on her first admission (discounting the penicillin) does not rule out pernicious anaemia, since it is known that marmite, given in sufficient quantities, may contain enough folic acid to bring about a remission of that disease. As a case of pernicious anaemia was not available here to feed on gastric juice from the patient, after incubation with beef, we were unable to ascertain whether our patient actually contained intrinsic factor. The absence of neurological symptoms in this case, which had relapsed so frequently, is also perhaps against a diagnosis of pernicious anaemia, but we think the question should remain open for the present. In any event we are here only concerned with reporting the response to penicillin.

Work is continuing on the treatment of these megaloblastic anaemias with penicillin alone, and if it can be confirmed that a haemopoietic response similar to that outlined above can be obtained, then we think the field of discussion on the origin of this type of anaemia will be greatly widened.

Summary

A case of megaloblastic anaemia is reported that responded to the administration of 400,000 units of crystalline penicillin G daily for seven days. Her reticulocytes rose to 37%, and this was followed by a rise in the haemoglobin and red blood cells. No other treatment was given.

The megaloblasts had disappeared from the marrow by the fifth day of treatment and the giant stab-cells had become very much less numerous.

The response obtained to penicillin in this case in no way differed from that obtained from potent liver extract, folic acid, B₁₂, or marmite.

It is suggested that the penicillin may have produced its effects by (a) destroying some organism which was competing for haemopoietic substances; (b) removal of some haemopoietic antagonist; (c) preventing the absorption of glutamic acid by competing organisms, which might thus interfere with the synthesis of pteroylglutamic acid; (d) affecting the metabolism and excretion of some essential amino-acids.

We are indebted to the Director of Medical Services, Kenya, for permission to publish this work, and for innumerable facilities granted to us at the Medical Research Laboratory and the Nairobi Group Hospital. To the European and African nursing staff of the Group Hospital we must also tender our thanks for many facilities to meet our somewhat fastidious demands.

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The first two women doctors since 1945 to volunteer and be accepted for the U.S. Army Medical Corps reported for duty in January in the preventive medicine division of the Office of the Surgeon-General. The original law authorizing the commissioning of women doctors ended in 1947, and the new authority was established in August last. During the war of 1939-45 the U.S. Army commissioned 72 women as physicians, and 26 of these served overseas. Their duties and conditions of service are identical with those of men except that they do not serve in "forward medical installations in combat zones."

TRIAL OF A CINCHONINIC ACID DERIVATIVE IN SOME COLLAGEN DISEASES

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[WITH SPECIAL PLATE]

When investigating a series of antidiuretic compounds Blanchard, Dearborn, Maren, and Marshall (1950a) suggested that a cinchoninic acid derivative, 3-hydroxy-2-phenylcinchoninic acid (H.P.C.) might be effective in treatment of conditions responding to A.C.T.H. This was prompted mainly by their discovery that when the pituitary was intact H.P.C., like A.C.T.H., reduced the ascorbic acid content of the rat adrenal.

After it had been shown by experiment on animals that toxicity was minimal (Marshall and Dearborn, 1950) a clinical trial of this preparation was made on patients suffering from conditions thought likely to benefit from a drug with A.C.T.H.-like properties (Blanchard, Harvey, Howard, Marshall, Newman, and Zubrod, 1950b). The cases treated were 10 patients with acute rheumatic fever, 10 with the chronic variety of rheumatoid arthritis, three with bronchial asthma, and two with disseminated lupus erythematosus.

The drug was administered by mouth for periods up to 21 days, the dose being 20 mg. per kg. of body weight on the first day, and 10 or 20 mg. per kg. each day or alternate days thereafter.

Toxic effects, except for one instance of drug sensitivity (fever), were nausea, vomiting, and abdominal cramps, limited to less than 10% of patients. To obviate these effects Blanchard *et al.* (1950b) advocated that the drug should be taken after breakfast in three doses at hourly intervals with an equal amount of sodium bicarbonate. The alimentary symptoms tended to disappear with continued use of H.P.C. and the necessity to withdraw the drug did not arise.

A supply of H.P.C. was made available to Professor J. W. McNee by Roche Products, Ltd. The drug, a yellow powder, was put up in coated tablets because of its bitter flavour. The dosage used by us was 20 mg. per kg. of body weight by mouth daily, which in some instances was increased to 40 mg. In seven of the twelve cases treated administration was continued for one week, in the rest for two or three weeks. The plan of administration recommended by Blanchard *et al.* (1950b) was followed, but sodium bicarbonate was not given.

The patients treated so far comprise four with rheumatic fever, two with polyarteritis nodosa, three with scleroderma, and three with lupus erythematosus.

Rheumatic Fever

Four patients—three with acute and one with sub-acute rheumatic fever—were treated with H.P.C. A typical response is shown in the Chart.