RESPONSE OF THE MEGALOBlastic ANAEMIAS OF PREGNANCY TO ANIMAL PROTEIN FACTOR

PRELIMINARY REPORT

BY

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In previous communications we reported the response of the megaloblastic anaemias of pregnant and non-pregnant African women to injection of crystalline penicillin G (Foy *et al.*, 1951a, 1951b, 1951c). Continuing this work in the treatment of the megaloblastic anaemias of Africans, we are now able to report the response of the megaloblastic anaemias of pregnancy to A.P.F. (animal protein factor of Lederle, now known as "aurofax"). This substance is a fermentation product of *Streptomyces aureofaciens* and a by-product of the manufacture of "aureomycin." It contains at least four factors in each 250-mg. capsule—namely, A.P.F., 6.7 mg. of aureomycin, 1 μ g. of vitamins B_{12a} and B_{12b}, and an "unknown factor."

Our patient took by mouth nine capsules a day. She thus received, in addition to the A.P.F. and unknown factor, 60 mg. of aureomycin and 9 μ g. of vitamins B_{12a} and B_{12b} daily. On the seventh day of treatment there was a maximum reticulocyte response of 27%, followed by adequate red-cell and haemoglobin regeneration. No other treatment but A.P.F. was given. The case has been followed up for three months and there has been no relapse. The response was similar to that obtained in other megaloblastic anaemias with liver, folic acid, vitamin B₁₂, or penicillin.

Case History

The patient was a five-months pregnant African about 30 years old, belonging to the Mita tribe. She was well nourished and of good physique, but said she never ate meat. She entered the King George VI Hospital, Nairobi, on May 1, 1951, for weakness, breathlessness, palpitations, and pains all over the body. She was icteric and obviously ill. There was no oedema of the back or legs. The spleen was I (Hackett). The liver was not palpable. The heart showed nothing that was not compatible with her severe anaemia. The chest was normal, and she was afebrile. Her tongue, teeth, and nails were all normal, and she had no nervous symptoms. Free acid was present in the gastric juice after histamine. She had had three previous pregnancies: all the children had died between the ages of 2 months and 1 year. Scanty hookworm eggs were found in the faeces.

Laboratory Examination on May 2 showed: red blood cells, 1,310,000; haemoglobin, 4.9 g.; haematocrit, 15.7%; reticulocytes, 2.8%; M.C.V., 120 μ^3 ; M.C.H., 37 μ g.; M.C.H.C., 31.4%; Schumm's test, +; indirect Van den Bergh, 2.6 mg. per 100 ml.; sickling tests, negative; white blood cells, 6,000; sternal marrow (Arikin's method), hyperplastic, many giant stab-cells and intermediate megaloblasts; Price-Jones curve, slightly to right of normal; macrocytosis, 14.2%; microcytosis, 0.0; M.C.D., 7.87 μ ; M.C.A.T., 2.43 μ . The rest of the blood findings for her stay in hospital are shown in the Chart.

From May 2 to 9 she was kept under observation and no treatment whatever was given. During this period the reticulocytes fluctuated between 1 and 5.4%. The blood count and haemoglobin fell slightly. On May 9 750 mg. of A.P.F. was given by mouth, and on the following 13 days 2.25 g. (nine capsules daily). During the next 14 days the amount was reduced, owing to short supply, to 1.5 g. daily. Five days after the beginning of treatment there was a great improvement in the patient's appetite and she said she felt much better.

Seven days after the first dose of A.P.F. the reticulocytes rose to 27%, the expected level for the blood count. On May 22 the red cells had risen to 2,192,000, the haemoglobin to 7 g., and the haematocrit reading to 25.5%. About this time the patient complained of belly-ache, but otherwise felt well and was walking about. She left the hospital on June 28, with a red-cell count of 3,420,000, a haemoglobin of 10.5 g., and a haematocrit of 35%. Sternal puncture on June 25 showed an active erythroblastic-normoblastic marrow with no megaloblasts or giant stab-cells. The indirect van den Bergh was still 2 mg. per 100 ml., and Schumm's test continued to be positive. She attended as an out-patient on July 5, with a red-cell count of 3,750,000 and a haemoglobin of 10.8 g. On August 28 she again visited us, looking fit, with a red-cell count of 4,440,000 and a haemoglobin of 12 g. Neither the red blood cells nor the haemoglobin reached normal values: we suppose that here, as in Macedonia, the pregnant need treatment continually until the child is born.

Discussion

Animal protein factor has been shown by numerous investigators to be a growth factor for pigs (Cunha *et al.*, 1949), chicks (Stokstad and Jukes, 1950), turkeys (McGinnis *et al.*, 1949), and rats (Piccioni *et al.*, 1951). Penicillin, "terramycin," and "bacitracin" have similarly been shown to promote animal growth and/or reduce mortality in the newborn.

In some groups of animals A.P.F. is stated to produce its growth effects independently of vitamin B₁₂, and several investigators have shown that aureomycin has a "sparing action" on vitamin B₁₂ (Stokstad and Jukes, 1951; Cravioto-Munoz *et al.*, 1951). Reid and Couch (1950) failed to obtain growth increases in chicks which were fed A.P.F. that contained vitamin B₁₂ but no aureomycin, but growth was increased when the A.P.F. containing *Streptomyces aureofaciens* fermentation products was given. Colby and his collaborators (1952) have found that 100 mg. of aureomycin fed daily to lambs decreased their growth rates, but growth could be stimulated by the addition of the vitamin-B complex. Davis and Chow (1951) have found that aureomycin increases the vitamin-B₁₂ activity of faeces in rats and improves their nutritional status, and they suggest, as we did, that the antibiotic is selectively destroying certain micro-organisms which are competing for accessory factors synthesized by other groups of bacteria, or destroying organisms that produce toxins that might be interfering with the utilization of accessory nutrients.

Roine and Elvehjem (1950) have claimed to have improved the growth of guinea-pigs by adding gum arabic, potassium acetate, and MgO to the diet, and suggest that such a diet produces an environment in the caecum in which suitable "growth-promoting" flora can develop. Biely and March (1951) have found that small amounts of aureomycin added to a diet that is suboptimal in nicotinic and folic acids and riboflavin makes such a diet adequate for the growth of chicks. They suggest that this may be due to a reduction in the microflora of the gut which is competing with the host for members of the vitamin-B complex and/or to a proliferation of those micro-organisms which are synthesizing these vitamins (*cf.* Foy *et al.*, 1951a, 1951b, 1951c).

It has been suggested that the antibiotics that we have used in the treatment of the megaloblastic anaemias have produced their effects by virtue of some unknown haemopoietic factors which they contain, rather than to any effects that they may have on the gut flora. Although this may be the case with A.P.F., as pointed out below, it is not likely to be the case with penicillin. We have found that penicillin will produce its effects in the megaloblastic anaemias only if given in small doses—not more than 200,000 units twice daily by the intramuscular route, and not more than 125,000 units twice daily by the oral route. Given in greater amounts than these it is haemopoietically ineffective (Foy and Kondi, unpublished). If the effects of penicillin were due to its content of unknown haemopoietic factors then one would expect results from large as well as small doses.

Our explanation of these facts, provisionally, is that large doses of this antibiotic not only clear the gut of the microflora that is competing with the host for the supply of essential factors but also destroy that flora which is synthesizing these factors. We are aware that increases in the dosage of antibiotics does not necessarily widen their spectrum of action, but variations in bacterial sensitivity are known to exist and to be affected by varying doses of antibiotics. There is also the possibility that the antibiotics may be producing their effects by suppressing a flora responsible for an enterotoxaemia (Sieburth *et al.*, 1951).

If these antibiotics are producing their growth effects by dealing with bacterial flora, the possibility of the production of antibiotic-resistant strains should be borne in mind. This will be particularly the case if small continuous doses are given. It may well be that some of the discrepant results are due to production of antibiotic-fast competitor strains.

The response that we have obtained to A.P.F. in the megaloblastic anaemias of Africans may be due to (1) A.P.F., (2) unknown factors in the A.P.F., (3) the small amount of aureomycin in the A.P.F. acting directly on bacterial flora, or (4) small amounts of vitamin B₁₂ contained in aureofax and "spared" by the antibiotic present.

Summary

Megaloblastic anaemia of pregnancy in an African has been successfully treated with animal protein factor. A maximum reticulocyte response was attained on the seventh day of treatment, and was followed by the expected red-cell and haemoglobin regeneration and a return of the bone marrow to normal. No other treatment was given, and the response was similar in every way to that which we have obtained from liver, folic acid, vitamin B₁₂, and penicillin.

It is suggested that the response may have been due to (a) changes in the bacterial flora of the gut brought about by the antibiotic present in the A.P.F.; (b) an unknown haemopoietic factor present in the A.P.F.; (c) the small amount of vitamin B₁₂ present in the A.P.F.; or (d) "sparing action" of the aureomycin on the vitamin B₁₂.

The failure of large doses of antibiotics to produce a cure in the megaloblastic anaemias would seem to indicate that their effects are not due to their supposed content of unknown haemopoietic factors, but rather to their action on the microflora of the intestine.

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Onchocerciasis is found in East and West Africa, the Congo, and South America. The worst area is West Africa, where at present for every five persons with the disease two go blind. This year survey teams are being sent out to West Africa by the British Empire Society for the Blind to tackle the menace of onchocerciasis which is spreading along the river systems there. In the northern territories of the Gold Coast alone 100,000 people are believed to be infected and 40,000 of them are blind. The disease is depopulating the most fertile land—land scheduled for development. A recent report concerning this area tells how an ophthalmic surgeon visiting a village waited for patients to turn up. Eventually a long line of men and women—113 of them—shuffled slowly into view. As they came closer he was appalled to see that they walked patiently in single file, each with a hand on the next person's shoulder, because they were all blind or nearly blind. The British Empire Society for the Blind is confident, however, that further research into methods of elimination of the simulum fly and the testing and development of recently discovered drugs will eventually bring control of the disease. This work is part of the Society's widespread campaign against blindness and eye diseases in which over 20 colonial territories are already participating.