40 per cent hæmoglobin (Zeiss), V.I. 1.5, megaloblastic sternal marrow and less than 1 per cent reticulocytes; discharged on 29th January, 1941, with 5.1 mil. red cells and 102 per cent hæmoglobin after 6 c.cm. of reticulogen. Maximal reticulocytic response 8 per cent. Re-admitted on 27th August, 1941, with 2.6 mil. red cells and 70 per cent hæmoglobin. Two ounces daily of marmite from 3rd September, 1941, to 7th October, 1941, raised his red cell count to 4.8 mil. and hæmoglobin to 100 per cent by 7th October, 1941. Maximal reticulocyte response was 6 per cent. This patient gave no history of diarrhœa or of paræsthesiæ. He had free hydrochloric acid in his stomach juice.

Case 6.—A Hindu male, 25 years old, admitted on 19th December, 1940, with large, pale, frothy diarrhœa with very high total fat and free fatty acid content. Twelve c.cm. of anahæmin without restriction of diet raised his red cell count and hæmoglobin from 1.5 mil. per c.mm. and 40 per cent to 5.1 mil. and 100 per cent respectively. His V.I. on admission was 1.7 and sternal marrow showed a well-marked megaloblastic reaction. Maximal reticulocyte response was 14 per cent. Gastric juice contained free hydrochloric acid.

Case 8.—A Hindu male, 37 years old, admitted on 14th November, 1940, with foul, frothy and fatty (total and soaped fat and free fatty acid high) diarrhœa of six months, histamine-fast achlorhydria and well-marked megaloblastic marrow. Twelve c.cm. of anahæmin raised his red cell count from an initial 2.5 mil. per c.cm. to 5.2 mil. and hæmoglobin from 65 to 105 per cent on 29th January, 1941. Maximal reticulocyte response was 10 per cent.

Summary

Fifteen cases of nutritional macrocytic anæmia with and without diarrhœa are reported as having well responded to refined liver extracts of the anahæmin type.

It is therefore inferred that refinement of liver extract by saturation with ammonium sulphate does not affect its potency against nutritional macrocytic anæmia.

The mechanism of production of nutritional macrocytic anæmia may be by deprivation of hæmopoietin to the bone marrow by defective supply of Castle's food factor even as the genesis of pernicious anæmia is supposed to be by similar deprivation by defective secretion of Castle's gastric factor. There is no case for postulating a totally different mechanism for the two anæmias.

Practically all the hæmatological studies on the cases reported here were made by Lieut.-Colonel G. R. McRobert's senior house physicians at the time, by Dr. P. Ramachander in the earlier cases and Dr. V. S. Raghunathan in the later ones. The volume index and thrombocyte count determinations were made by the clinical pathologist of the hospital at the time. The biochemical investigations were made by the department of biochemistry of the Madras Medical College. I am grateful to them for their help. My indebtedness to Lieut.-Colonel G. R. McRobert, I.M.S., Professor of Medicine, Medical College, and Physician and Superintendent, General Hospital, Madras, for his constant drive and encouragement and helpful discussions cannot find adequate expression.

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TREATMENT OF BLACKWATER FEVER

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Ætiology.—Thirty-seven cases of blackwater fever are recorded. The condition was precipitated during the administration of quinine (18 cases), pamaquine (18 cases) and mepacrine (1 case).

In all cases there was history of malaria, one to five attacks during the twelve months preceding the attack of blackwater fever. Thirty-four cases were treated for malignant tertian malaria, and the remaining three cases for benign tertian malaria. In the latter three cases, during the particular bout of fever which was clinically benign tertian, malignant tertian rings were not demonstrated in the blood by careful examinations, but in two of them previous infection with

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Later communication

(*a*) Since sending the main article for publication, I have had a chance of studying two patients with sprue syndrome (diagnosis based on history, fat content of stools, flat glucose tolerance curve) with normoblastic bone marrow on admission. After failure of TCF liver extract and hydrochloric acid in full doses, in one of nicotinic acid, in the other of riboflavin, anahæmin produced striking and immediate lingual and gastro-intestinal remissions.

(*b*) Specific therapy in Addisonian pernicious anæmia is known to induce a reversion from megaloblastic to normoblastic marrow in 24 hours. I have recently studied in two cases the effect of anahæmin on the marrow of nutritional macrocytic anæmia. Two micro-photograph prints of sternal marrow smear are annexed, one made before and the other in 20 hours after the first 4 c.cm. of anahæmin given to a patient with nutritional macrocytic anæmia (*see* plate XIII, figures 1 and 2). I am grateful to Dr. D. Govinda Reddy, Professor of Pathology, Madras Medical College, for them. They speak for themselves of the striking reversion to the normoblastic condition in under one day. Davidson *et al.* (1942 : *Quart. J. Med.*, **11**, 19) have reported the commencement of such reversion in 6 hours in pernicious anæmia. They argue that such early reversion demonstrates easy convertibility of a megaloblast into a normoblast. It is out of place here to discuss fully the implications of this observation.

malignant tertian malaria was proved by the presence of characteristic gametocytes in the blood.

None of the patients were actually febrile with a malarial bout of fever. They were up and about taking antimalarial drugs and doing various grades of work, with the exception of one who was bed-ridden and three who were on a train journey.

Thirteen of the patients had been taking quinine as a prophylactic, and the rest were on treatment with the antimalarial drugs.

All the cases were recorded between October and March, with the exception of one in April.

Thirty-five of the patients were weak and debilitated, while two appeared to be in good health.

One had a positive W.R., and had taken one course of anti-syphilitic treatment.

Hæmolytic substances were demonstrated in the peripheral blood of three patients. Their presence was very transitory; they were found in samples of blood taken five minutes before other samples which showed early hæmoglobinæmia, but not in the samples taken ten minutes before that. In one of these patients who seemed to show three distinct attacks of hæmolysis within the next fifteen minutes, hæmolytic substances were demonstrated in two samples, 1 and 4, out of six samples of blood taken every two minutes during that period.

This probably indicates that during a clinically single attack of hæmolysis, hæmolytic substances are poured out in distinct gushes, and become almost immediately fixed to the red blood cells; in the above cases it must have been within five minutes. To get the hæmolytic serum, the red blood cells had to be centrifugalized off within two minutes, as after that it lost its hæmolytic properties, although hæmolysis continued in the test tube.

It was further observed that the serum had the following properties : 1. It hæmolyzed infected and non-infected red cells. It could not be proved that infected cells were more susceptible to hæmolysis. 2. Traces of quinine, 1 in 3,000, pamaquine, 1 in 10,000, and mepacrine, 1 in 500, accentuated the hæmolytic process; this proved that mepacrine could produce blackwater fever, but it does not do so often, because it required larger concentrations of it to help the process. 3. The hæmolytic process was prevented by the addition of antivenene, 1 in 300. Clinically a recurrence of hæmolysis did not occur after ninety-six hours in any of the control cases.

Treatment.—Antivenene was used to prevent recurrence of intravascular hæmolysis with absolute and definite results. Using 20 c.cm. of the concentrated serum intravenously at once, and repeating 10 c.cm. every four hours for the next seventy-two to ninety-six hours, it was found that a second attack of hæmolysis did not occur. Using three controls with a smaller dosage, the hæmolysis was found to be milder, and the patients were, as it were, allowed to have one to four attacks of hæmolysis, the maximum number recorded by changes in the colour of the urine being five.

The prevention of precipitation of acid hæmatin in the kidney tubules was ensured by taking the following precautions : by keeping the urine strongly alkaline throughout the twenty-four hours; by withholding the sodium chloride till the urine was alkaline; by not allowing the temperature of the patient to rise higher than 102°F.

Immediate alkalinization of the urine was acquired by giving 150 c.cm. of a 4 per cent sodium citrate solution intravenously, followed by one dram doses of sodium citrate two to four-hourly by mouth. Where vomiting did not allow the result to be achieved, a total of six to eight drams of sodium citrate could be infused intravenously by drip in twenty-four hours.

To ensure diuresis, glucose solution 25 per cent, 100 to 200 c.cm. with vitamin C, 100 to 200 mg. was given intravenously every four hours. Glucose saline was withheld during the early stages when the urine was acid, for the reasons given above. In cases with threatening anuria, sodium sulphate, 4.285 per cent, was an useful adjunct to glucose and vitamin C. Glucose also supported the circulation, and vitamin B 25 mg. in twenty-four hours was added.

Vomiting was checked by sodium citrate given by the mouth, and could be further relieved by an injection of atropine gr. 1/100 to 1/50 given intravenously. Vomiting usually disappeared twenty-four hours after an attack of hæmolysis, and was apparently caused by the irritation of the bile in the stomach (to distinguish it from the vomiting of later stages which may develop from kidney or liver failure). An automatic stomach-wash, produced by giving the patient a pint or more of a 2 per cent sodium bicarbonate solution and subsequent vomiting, gave immense relief in other cases. Calcium gluconate, 20 c.cm. of a 10 per cent solution, repeated if necessary in two to four hours, checked the vomiting in cases showing renal acidosis or alkalosis produced by severe vomiting, conditions in which there is a fall in the blood calcium. A very useful drink retained by many patients was made as follows : glucose dr. 2, sodi bicarb. dr. 1, and lemonade one bottle, given iced.

Blood transfusion was used in three cases in which hæmoglobin had fallen to 20 per cent. Reactions were prevented by the following precautions : by matching the blood under the high power of the microscope; by using 120 c.cm. of a freshly prepared 4 per cent sodium citrate solution for each 400 c.cm of blood; by collecting the blood freely at room temperature; by not shaking the blood and the citrate to ensure admixture as this is unnecessary; by using no warming apparatus before infusing the blood which was to be given at room temperature; by ensuring that the urine was alkaline. This latter

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NOTE ON THE TREATMENT OF ANGULAR CONJUNCTIVITIS WITH RIBOFLAVIN

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THE successful treatment by riboflavin of a number of cases of superficial keratitis was described in two previous papers (Aykroyd and Verma, 1942; Verma, 1942). In the course of this work it was observed that a number of patients with superficial keratitis, associated with angular stomatitis and other signs of riboflavin deficiency, were also suffering from angular conjunctivitis, and that the latter condition tended to clear up on the administration of riboflavin. Further investigations on the effect of riboflavin therapy in cases showing angular conjunctivitis were then undertaken. These investigations were interrupted by Army service, and since it will probably be some time before the work can be resumed, the preliminary observations are here recorded.

Angular conjunctivitis and the Morax-Axenfeld bacillus.—This diplo-bacillus was described in 1896 by Morax and Axenfeld simultaneously (Duke-Elder, 1938). It is found in abundance in smears from cases of angular conjunctivitis. It is a saprophyte and is said to produce conjunctivitis by excreting an exogenous protein-dissolving ferment, which acts by macerating the epithelium. The specific treatment of angular conjunctivitis associated with this bacillus is by drops of zinc sulphate solution, which banishes the symptoms rapidly. The action of zinc, according to Duke-Elder (*loc. cit.*), is not a bactericidal one, for the diplo-bacillus grows well in a culture-medium containing zinc; the zinc acts by inhibiting the proteolytic ferment secreted by the bacillus and so rendering it impotent.

Effect of riboflavin therapy.—Smears from cases of angular conjunctivitis associated with signs of riboflavin deficiency showed abundant Morax-Axenfeld bacilli (MAB). On treatment by the administration of riboflavin, the angular conjunctivitis disappeared and the smears became negative. No local treatment was given. Twenty cases were treated, 12 males and 8 females. Of these, 2 showed superficial keratitis and 14 had other signs of riboflavin deficiency, *i.e.* angular stomatitis and sore and fissured tongue. Some of the male patients showed an eczematous condition of the skin of the scrotum, characteristic of riboflavin deficiency. In only 3 cases was angular conjunctivitis present without other signs of riboflavin deficiency. Some typical cases are described below :—

(1) F. 30. Complained of photophobia, itching, burning, watering and discharge from the eyes for a period of 6 weeks. Examination showed angular conjunctivitis and superficial keratitis. Conjunctival smears were highly positive for MAB. Three mgm. of riboflavin were given by intramuscular injection daily for 7 days. All symptoms and signs disappeared, and the conjunctival smear became negative.

(2) F. 11. This patient complained of pain, watering and itching in both eyes for 10 months. She had xerosis conjunctivæ, angular conjunctivitis, corneal 'stippling', angular stomatitis and fissured tongue. The conjunctival smear was positive for MAB. After the intramuscular injection of 4 mgm. of riboflavin daily for 5 days the eye symptoms and signs cleared up, and the smear became negative.

(3) F. 30. Had angular conjunctivitis, superficial keratitis and angular stomatitis. Duration over 3 weeks. Treatment with riboflavin, 5 mgm. daily for 8 days by mouth, was followed by relief of eye signs

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precaution was also important because the infused blood was equally liable to hæmolysis.

Blood was still positive for malignant tertian malaria in seventeen cases after the hæmolysis. No antimalarial drugs were used. A low-grade pyrexia persisted in thirty-one cases from three to twelve days, but probably it was due to the same causes as the fever in anæmia, as it was still present when the serum showed no evidence of hæmoglobinæmia. However it would appear that antimalarial drugs could be used if antivenene was given at the same time, but this was not tried in any of the cases.

Secondary anæmia was treated with fersolate, vitamin B, vitamin C, and sometimes by the use of crude liver extracts given by injection if the response to the other drugs was not adequate. Cod-liver oil was given to all debilitated cases, and a generous diet containing chicken, fish, eggs, fruit, milk and vegetables was given from the very start, as soon as the vomiting stopped. Convalescence was rapid.

Result.—Thirty-six cases were treated with antivenene, and all recovered. One case not treated with antivenene, as it was not available, died in spite of all other measures after three attacks of hæmolysis. No kidney or liver complications were recorded in any of the thirty-six cases. No stimulants were used. Oxygen was given to very anæmic cases, and the circulation was supported by glucose till blood transfusion was given. No antimalarial drugs were given to any of the cases after the attack of hæmolysis was over, although some of them still showed parasites.

Conclusions.—1. Hæmolytic substances are responsible for an attack of blackwater fever. Their presence is transitory and therefore difficult of demonstration, but by well-timed examination they can be demonstrated.

2. Antimalarial drugs accentuate the hæmolytic process. Mepacrine does so only in greater concentration, and hence blackwater fever seldom occurs with mepacrine.

3. Antivenene neutralizes the effect of hæmolytic substances in blackwater fever, and so is very useful in preventing further hæmolysis. Its use during the administration of antimalarial drugs to blackwater fever cases has however not been tried.