In its future work W.H.O. will be guided by these important observations, and it is believed that the results of mass-treatment campaigns will be improved thereby. The ultimate success, however, must depend on how the gains achieved by the mass-treatment campaigns are consolidated by the expanding health services of the local health administrations.

Conclusions

Recent advances, particularly the development of effective "one-shot" or "few-shots" forms of therapy with P.A.M. (procaine penicillin in oil with 2% aluminium monostearate) have made possible rational and more readily carried out mass campaigns against the treponemal diseases. In these campaigns there is an important role for international organizations such as W.H.O. and Unicef.

To make comparative evaluation studies possible, and to be fully effective in large-scale campaigns, preparations of P.A.M. must give prolonged serum concentrations. Constant vigilance is necessary to ensure that suitable preparations are used, since there are enormous differences in the products available in different countries to-day.

In carrying out mass treponemal disease-control campaigns it is desirable to maintain primary control areas in which serological tests are used systematically. In the broader programme it is necessary to have as full a coverage of the population as possible and to treat routinely the contacts of infectious cases.

Mass treatment alone is not enough. The gains obtained by mass therapy must be firmly consolidated. In order to do so, re-surveys of the entire population should be carried out at periodic intervals, which should be not less frequent than 6-12 months for several years.

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PROGUANIL AND BLACKWATER FEVER

BY

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Proguanil, unlike quinine, pamaquin, and to a lesser extent mepacrine, has not been known to precipitate blackwater fever when administered to malarial patients. Two cases following its use are therefore worth recording.

Case 1

A civilian aged 20 was admitted to a military hospital on November 1, 1949. He hailed from the Punjab, and had entered a locally endemic area about nine months previously. There was no history of malaria, but symptoms suggestive of latent malaria were present for about three months before admission.

At the time of admission he had been suffering from malarial agues for two days. His temperature was 101-103.8° F. (38.3-39.9° C.), pulse 80-96, and respirations 20-26. His blood showed a few M.T. rings. He was put on a routine proguanil course of 200 mg. thrice daily for two days, followed by 100 mg. thrice daily for eight days. The response to proguanil was good, and next day there was profuse sweating and his temperature started falling. After 800 mg. of the drug had been administered he had repeated rigors again and passed 240 ml. of characteristic very dark haemoglobinuric urine. He felt sick and vomited thrice, and then followed prostration, giddiness, diminished vision, and palpitation on slight exertion. His blood pressure was 110/70 mm. Hg. There was pain in the loins and urgency to micturate, but only a few drops of urine were passed, sometimes none. Temperature 99.4° F. (37.4° C.), pulse 106, and respirations 24.

Laboratory Investigations.—Urine: heavy cloud of albumin, trace of sugar, considerable amorphous deposits, and few hyaline casts, and Hb spectroscopically. Blood: Hb, 60% (Sahli); red cells, 3,200,000 per c.mm.; white cells, 13,900 per c.mm. (polymorphs 54%, lymphocytes 40%, mononuclears 4%, eosinophils 2%). Blood urea, 43 mg. per 100 ml.; urine urea, 4.9 g. per 100 ml. Blood slides negative for malaria parasites; serum positive for oxyhaemoglobin and methaemoglobin (methaemalbumin not tested for); blood collected for serum bilirubin haemolysed; total serum proteins, 7.2 g.% (albumin 4 g.%).

Alkalis at the rate of 120 gr. (8 g.) in 24 hours were given by mouth and 3 pints (1.7 litres) of 5% glucose-saline was given in 12 hours by intravenous drip. In about 24 hours there was no vomiting, and the urine, which had begun to be passed in increasing quantities, showed signs of progressive clearing. The patient, however, had another but milder rigor and the urine became darker again. An intravenous injection of 20 ml. of concentrated (anti-snake) antivenene was given to prevent further haemolysis. Next day diuresis

set in and the urine cleared rapidly. Subsequently the average daily urine output was 2.91 litres against an intake of 3.24 litres, and the general condition improved concomitantly. Increased urobilin was present in the urine for five to six days, and a few M.T. gametocytes were found in the peripheral blood on daily examination. On the eleventh day the patient was up and about and feeling all right. His Hb was 70%, red cells 3,890,000 per c.mm., and the urine normal except for a trace of albumin. On the fourteenth day he was discharged.

Case 2

A man aged 30 was admitted to a military hospital on September 17, 1952. He had taken 600 mg. of proguanil on his own for an attack of malaria, and had developed a mild attack of blackwater fever about 8½ hours later. He was a Punjabi and had been in the locally endemic area for about a year. His last attack of malaria occurred in the North-west Frontier Province in 1942.

On admission he complained of fever with rigor since noon that day, and headache, weakness, and lumbar pain since about eight and a half hours after taking proguanil. Two hours later he vomited twice and then passed a small quantity of urine, but did not notice anything abnormal about it in the darkness of the night. About six hours later in the morning he passed about 4 oz. (114 ml.) of dark haemoglobinuric urine. Urine was passed in increasing amounts subsequently and cleared in about 24 hours. No facilities were available here to do any special examinations. A routine urine deposit examination, however, proved that the case was not one of haematuria and a blood examination showed B.T. rings.

Four days later, when the patient was received at the main hospital, the serum bilirubin was 0.4 mg. per 100 ml., both serum and urine were negative for oxyhaemoglobin and methaemoglobin, but the urine was positive for urobilin in 1 in 80 dilution. The blood count was about normal. A few B.T. gametocytes were present in one sample of blood. Treatment consisted of adequate quantities of alkalis and fluids by mouth.

Discussion

It is an undisputed fact that blackwater fever sometimes develops in endemic areas in individuals who have never taken any antimalarial drug. Although it is malaria which is the chief factor in the aetiology of blackwater fever, any antimalarial drug can, however, be a precipitating factor, quinine being the most dangerous of them all. The exact mechanism by which they precipitate the disease is not known. But in relation to current theories regarding mechanism of haemolysis in blackwater fever the following are the possibilities.

1. *Blackwater fever is a sensitization reaction to proteins of malaria parasites. Antimalarial drugs destroy malaria parasites and liberate proteins which are required to precipitate a haemoclastic crisis.*—Increased haemolysis seems to be a natural process during the acute stages of malarial infection, as shown by increased serum bilirubin figures obtained by several workers. The haemolysis bears no relation to the degree of infection, and lighter infections may be associated with heavier bilirubinaemia, but apparently it is heavier in cases with splenic enlargement. The parasite is therefore not directly responsible for the haemolysis. Plehn (1936) suggested that the red blood cells become hypersensitive to the proteins of the malaria parasites which are liberated, with their repeated destruction during each attack of clinical malaria, and that a stage in hypersensitiveness is finally reached when the liberation of a little of this protein precipitates the haemoclastic crisis of blackwater fever.

Fernán-Núñez (1936) assembled evidence in support of Plehn's theory. He found that 16 out of 410 individuals who had lived in an endemic area for six months or more and who had suffered from M.T. malaria were sensitive to an antigen prepared by him from malaria parasites, and that blackwater fever did not occur when these individuals were removed from the site. More recently, Findlay and Markson (1947), with their experience of troops in Africa, have again suggested that sensitization to the malaria parasite is an essential cause of haemolysis in blackwater

fever. However, although all antimalarial drugs destroy malaria parasites, and red blood cells containing them constantly undergo lysis and must liberate this hypothetical haemolytic substance, quinine is the most predisposing drug. Hence such a sensitization reaction alone cannot explain the haemoclastic crisis of blackwater fever.

2. *Blackwater fever is the result of autohaemolysis. These autohaemolysins can accumulate in the spleen and can be thrown out into the general circulation by the contractile action of antimalarial drugs on the spleen. Antimalarial drugs also help in the production of autohaemolysins.*—Gear (1946) has suggested an explanation for the origin of haemolysins in blackwater fever. On the analogy of experimental evidence obtained by him and by Schwenker and Compoier (1939) he thinks that red blood cells infected with malaria parasites become auto-antigenic and give rise to haemolytic auto-antibodies in the reticulo-endothelial system. The haemolytic auto-antibodies sensitize red blood cells, infected or non-infected, and the sensitized cells can undergo haemolysis in the presence of the complement. He further suggests that antimalarial drugs may help in the production of auto-antigens.

The works of Barcroft (1920), Knisely (1934), Fåhræus (1939), and Ham and Castle (1940) suggest that the spleen is an ideal place for sensitization of red blood cells described by Gear. The haemolytic auto-antibodies over and above those required to sensitize red blood cells in the spleen accumulate there and can be thrown out into the general circulation when the spleen contracts. A haemoclastic crisis may then follow. It is interesting to note that quinine causes splenic contraction. The soft, enlarging, and congested spleen of acute and subacute malaria can alone undergo contraction. Thus blackwater fever does not usually occur in cases of chronic malaria with large fibrous non-contractile spleens. The action of other antimalarial drugs in causing splenic contraction has apparently not been described.

It may be added that Butts (1945) thinks that the sensitizing agent in malaria parasites is an Rh-like substance. Iso-immunization against it causes haemolysis in blackwater fever.

3. *Blackwater fever is the result of imbalance between lytic agents and their inhibitors. Antimalarial drugs inactivate inhibitors.*—Lytic agents in tissues have been reported by Maegraith, Martin, and Findlay (1943), Ponder (1944), Brown and his colleagues (1944), and Brückmann and Wertheimer (1945), and in the blood by Laser and Freidmann (1945). Maegraith and his colleagues suggest that the natural haemolysis which occurs normally in the body depends on the balance between the lytic agents and their inhibitors. They found that sera of blackwater fever cases during a haemolytic crisis and the sera of cases of acute malaria were less potent in their content of inhibitors than the sera of normal persons. It seems that in blackwater fever the precipitating factors directly or indirectly bring about a reduction or absence of the inhibitors.

Laser and Freidmann (1945) isolated a haemolytic substance from normal human plasma. More recently Laser (1948) has found the same substance in much higher concentrations in the blood of monkeys heavily parasitized with *Plasmodium knowlesi*. The parasites seem to produce it or cause its appearance in the infected cells, which are destroyed when its concentration is high, and the substance is thus released into the plasma to increase the concentration of lytic agents.

Nocht and Kikuth (1929) found that haemolytic amboceptors produced in rabbits by the injection of dog's blood have an increased haemolytic effect *in vivo* but not *in vitro* if quinine is simultaneously or subsequently injected. It was found that small amounts of haemolytic amboceptors—not enough to produce even haemoglobinaemia—produced haemoglobinuria when given with quinine. It therefore seems that quinine is capable of suspending or at least considerably weakening the neutralizing effect of inhibitors *in vivo*.

4. *Blackwater fever is caused by acidosis. Acid salts of quinine especially predispose.*—Any suggestion that acidosis may play a part in haemolysis *in vivo* in blackwater fever is difficult to contemplate, as acidosis associated with changes in the pH of blood is incompatible with life. Acid salts of quinine, however, especially seem to predispose to blackwater fever. *In vitro*, sodium bicarbonate inhibits haemolysis by antimalarial drugs.

5. *Antimalarial drugs directly aid haemolysis in blackwater fever.*—Zylmann (1944) found that a solution of quinine had a haemolytic effect on red blood cells from a normal as well as from a malarial subject, but mepacrine acted more vigorously on cells from the latter. Birnbaum and his colleagues (1945) found that, *in vitro*, quinine was haemolytic in a dilution of 1 in 200. When quinine, mepacrine, or pamaquin was not haemolytic in a given concentration, addition of bile salts precipitated haemolysis, but

sodium bicarbonate exerted a delaying effect. Singh and Singh (1944) found that quinine 1 in 300, pamaquin 1 in 10,000, and mepacrine 1 in 500 accentuated the haemolysis caused by serum obtained from blackwater fever cases.

In vivo, Ross (1927) found that malarial patients treated with quinine or pamaquin show initially an increased bilirubinaemia. It is highest about the first day and takes a few days to return to normal. Hughes and Shrivastava (1930) noticed a similar effect within twenty minutes of an intravenous injection of quinine.

Antimalarial drugs may therefore directly aid the haemoclastic crisis in blackwater fever.

Use of Antivenene.—Antivenene apparently helps in controlling haemolysis in blackwater fever (Singh and Singh, 1944, 1945, 1946; Tandan, personal communication). High doses prevent a second attack, and with smaller doses the haemolysis can be milder. Along with its use, mepacrine and quinine, even in full doses, were found to be safe in treating the residual malaria when necessary. Ponder's (1944) finding that the inhibitors present in tissues were not species-specific offers a probable explanation of the beneficial effect of antivenene. Its use supplies non-specific inhibitors. The dose of antivenene required would apparently depend on the balance between concentration of haemolysins and their inhibitors in the blood. Judging from clinical results and from the analogy of the action of antidiphtheritic serum, it is probable that the concentration of antivenene falls to 50% of the original in three to four days and gradually to zero in 10 to 14 days.

Summary

Two cases of blackwater fever due to proguanil are described. The role of antimalarial drugs in precipitating haemolysis and the role of antivenene in its prevention are discussed.

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A description of the Singapore Medical Plan is given in the recently published "Annual Report of the Medical Department of the Colony of Singapore" for the year 1951. The plan envisages the modernization and enlargement of all the existing hospitals, the building of a new hospital centre, the expansion of the Tan Tock Seng Hospital to an 800-bed tuberculosis centre, a modern settlement for lepers, improved facilities for the treatment of mental diseases and mental deficiency, a chain of maternity and child welfare clinics throughout rural Singapore, a modern quarantine station, and an improved school medical and dental service. The report states that the plan seeks to double the present facilities in all medical fields, and "will take us quite a long way on that road to a free service for all, so ardently desired by so many of the community."

RENAL EXCRETION OF UREA IN THE WEST AFRICAN

BY

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During the course of a metabolic investigation on apparently healthy male West Africans low rates of urea elimination were consistently encountered despite the fact that the subjects were living on an *ad lib* self-selected diet apparently adequate in protein (about 70 g. a day, one-third of which was meat) and calories. It was decided, therefore, to investigate, on a somewhat larger series of subjects, the "normal" level of urea output and the output following the oral administration of urea.

Methods

Ten male West Africans, all apparently healthy and with no history of renal disease, acted as experimental subjects. Five Europeans, resident in the unit, were also investigated simultaneously with the Africans in order to provide a comparison with European figures determined under the same climatic conditions.

The subjects reported to the laboratory at 8 a.m., after their usual morning meal, and remained until the end of the investigation either sitting at rest or lying on a bed. Urine was collected hourly for three hours, during which time no fluid was taken. At the end of this period the urine flow was regularly of the order of 1 ml. a minute. It is customary in conducting urea-concentration tests in a temperate climate to withhold fluid from the subject for a period of eight to twelve hours in order to ensure a high degree of concentration of the urine (Harrison, 1937). In a tropical environment, however, a period of three hours' water deprivation seems to lead to an adequate reduction of urine volume.

At the end of this preliminary three-hour period the bladder was emptied and collections were made every hour for a further period of three hours. At the end of the first hour 20 g. of urea dissolved in 100 ml. of water flavoured with orange squash was administered by mouth. In this way a sample of the "resting" rate of excretion was obtained, together with two-hourly samples following the administration of urea on which could be estimated the maximum concentration achieved and the total excretion during the period. The urine samples were analysed for urea by the method of Karr (1924), the final colorimetric determination being performed in the Coleman junior spectrophotometer set at a wavelength of 5,000 Å.

Results.—The results obtained are set out in the Table.

It will be observed that in the case of every parameter the European values are higher than those obtained on the African subjects and are in tolerable agreement with the commonly accepted "normal" values for the European investigated in a temperate climate. The differences between the mean values for the two groups are statistically significant ($P < 0.01$).

Discussion

The fact that the African shows a low resting rate of urea excretion might at first examination be held to be due to a low dietary protein intake. Such data as are available, however, fail to support such a suggestion; the output of urea appears to be equally low in subjects living on a diet which they themselves have to buy and in subjects living on a diet provided by the laboratory which certainly has an adequate protein content. Even when a load of urea is presented to the kidney the African fails to excrete as much as the European; the maximum concentration achieved by the African subject is at times so low as to