1971; Slapak *et al.*, 1971) and our own findings make it possible to construct a probable sequence of events in the hyperacute rejection process. The first change appears to be damage to the capillary endothelium presumably by an antibody antigen complex with subsequent binding of complement. This is followed by platelets adhering to the damaged endothelium and subsequent aggregation in the glomerular and peritubular capillaries. Substances released from these damaged platelets could result in the intense vasoconstriction, which together with the platelet plugs themselves could cause acute haemostasis and immediate rejection. Thrombosis is probably a later and secondary change.

The action of heparin in prolonging graft survival is probably due to its direct effect on the platelets. This is consistent with our findings of a reduced fall in the platelet count in the heparin-treated dogs. Linn *et al.* (1971) suggested that substances released by the breakdown of complement contribute to the vasoconstriction during acute rejection. For this reason we established that our heparin preparation was free from anti-complementary activity. Modification of the rejection process with aspirin or cyproheptadine hydrochloride (Periactin) could also be explained by their antagonism to platelet aggregation (O'Brien, 1968; Burrows *et al.*, 1970).

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Amodiaquine-induced Agranulocytosis: Toxic Effect of Amodiaquine in Bone Marrow Cultures in Vitro

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Summary

A case of agranulocytosis is reported in which amodiaquine, to which the patient had been exposed, was found to be toxic to the patient's bone marrow cells when these were cultured in an agar colony system in vitro. This technique should be investigated in other patients with agranulocytosis as a possible means of detecting toxic agents.

Introduction

In most cases of drug-induced blood dyscrasias identification of a causative agent can be made only from the patient's history. This is relatively easy in cases of single drug exposure but more difficult when several drugs are involved. Re-exposure of the patient to the drug cannot be justified as a diagnostic procedure, and firm proof of the harmful nature of a drug has until now been difficult to obtain. This paper reports a case of amodiaquine-induced agranulocytosis in which the toxic effect of amodiaquine was shown by its inhibition of colony growth of the

patient's bone marrow cells in vitro. Although the technique of bone marrow culture is well established in the study of granulopoiesis, we are not aware of any previous reports of its use in the investigation of a patient with agranulocytosis.

Case Report

A 26-year-old woman visited New Guinea for a two-week holiday in July 1972. Antimalarial prophylaxis consisting of amodiaquine (Camoquin) 300 mg once a week was started four weeks before her departure and continued during and after her holiday (see chart). She took a total of eight doses (2.4 g), the last one three days before her admission. She was febrile and unwell for two days after the second dose, and had diarrhoea for one day on her return, but had otherwise experienced no untoward symptoms. One week before admission she was vaccinated, and immunized against typhoid and cholera. Two days before admission she became febrile, with myalgia and night sweats. Her local doctor prescribed tetracycline and Actified (triprolidine hydrochloride and pseudophedrine hydrochloride). When a blood count showed agranulocytosis, she was admitted to hospital.

She gave a history of five episodes of respiratory tract infection in the 18 months preceding admission, and she had noted increas-

Clinical course before and after admission. Vaccination against smallpox, typhoid, and cholera, and the duration of patient's stay in New Guinea are shown. Note the expanded time scale after admission.

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ing lethargy before going on holiday. Drug therapy other than amodiaquine was: erythromycin or penicillin for respiratory tract infections, oral contraceptives for eight months until one month before admission, and occasional chlorthalidose (Librium). Her work as an occupational therapist brought her into intermittent-contact with a variety of paints and glues.

On admission to hospital she was febrile (39.8°C), with cellulitis of the right great toe, and a bruise over the right shin. The white cell count was 1,000/mm³ (lymphocytes 96%, monocytes 4%), the haemoglobin concentration was 14.0 g/100 ml, and platelets were plentiful. Bone marrow aspiration yielded hypocellular fragments in which the only myeloid cells were a few myeloblasts and promyelocytes (see table I). *Staphylococcus aureus* and a group A haemolytic *streptococcus* were cultured from the lesion on the toe, *pseudomonas aeruginosa* was cultured from the sputum, and *streptococcus viridans* from one blood culture.

The patient responded rapidly to treatment in a low pathogen area with systemic antibiotics and leucocyte infusions from a histocompatible sister. Her fever resolved by the seventh day, at the same time as her blood neutrophil count began to rise (see chart). Serial bone marrow aspirations showed the progressive appearance of mature myeloid precursors, with an essentially normal marrow on the 11th day (table I). Her recovery was complicated by the development of a deep venous thrombosis on the seventh day, but this resolved satisfactorily with heparin therapy.

TABLE I—Bone Marrow Findings

Days after admission:	1	3	6	11
Cellularity*	+	++	+	+++
Differential (% of total cells):				
Blasts	12	11	5	1
Promyelocytes	1	10	8	5
Myelocytes	0	0	1	13
Metamyelocytes	0	0	1	19
Mature neutrophils	0	0	1	34
Eosinophils	1	2	0	0
Lymphocytes	63	41	39	12
Monocytes	5	6	9	2
Reticulum cells	2	0	0	0
Plasma cells	3	4	2	1
Erythroid precursors	13	26	34	13

* + = Grossly Hypocellular. ++ = Moderately hypocellular.
+++ = Normocellular. Megakaryocytes were plentiful in all samples.

MARROW CULTURES

Bone marrow aspirated on the eleventh day was cultured in semi-solid agar on feeder layers of peripheral blood leucocytes by the method of Robinson *et al.* (1970). Amodiaquine was added to the overlying bone marrow cell suspension in final concentrations of 0.005, 0.05, or 0.5 µg/ml, and triplicate cultures were established on feeder layers derived separately from five normal subjects. Control cultures, set up at the same time using marrow obtained from a haematologically normal subject, were treated identically. After 14 days' incubation, cultures were scored by counting the number of colonies containing more than 50 cells. Cell morphology was examined after staining with aceto-orcein.

Results

In our experience of 46 normal human marrows the mean colony count has been 64 ± 40 colonies per 2×10^5 cells plated (mean $\pm$ standard deviation of an individual observation). The results

obtained in both the patient and the normal control, in the absence of amodiaquine, were within normal limits (table II).

In the normal control amodiaquine-treated cultures formed significantly greater numbers of colonies than did untreated cultures (table II).

By contrast, colony formation by the patient's bone marrow was significantly inhibited by a concentration of 0.5 µg/ml of amodiaquine, the effect being uniform for each of the five feeder layers used. The colonies formed were also smaller in size than normal, but no morphological abnormalities were discernible in aceto-orcein-stained preparations. Lower concentrations of amodiaquine (0.005 or 0.05 µg/ml) had no significant effect on colony formation by the patient's marrow.

Discussion

Of the 4-aminoquinolone antimalarials, amodiaquine (Love *et al.*, 1953; Kennedy, 1955; Glick, 1957; Perry *et al.*, 1962; Booth *et al.*, 1967) and hydroxychloroquine (Polano *et al.*, 1965; Chernof and Taylor, 1968) have been reported to cause agranulocytosis. Most of the reported cases have been associated with ingestion of relatively large doses; in only two other cases has agranulocytosis occurred after the dosage recommended for prophylaxis (cases 3 and 4, Booth *et al.*, 1967).

The pharmacology of amodiaquine is similar to that of chloroquine (Love *et al.*, 1953; Goodman and Gilman, 1970; Grollman and Grollman, 1970). A plasma level of between 0.15 and 0.25 µg/ml would be expected after a single dose of 300 mg base, falling to between 0.02 and 0.04 µg/ml before the next dose one week later (Goodman and Gilman, 1970). In addition, leucocytes, which concentrate chloroquine (Goodman and Gilman, 1970), probably also concentrate amodiaquine. The highest dose tested in vitro (0.5 µg/ml) was selected to represent a possible bone marrow level after eight weeks' therapy, taking into account drug accumulation and concentration. The lower concentrations were included to test the possibility that the patient's cells were unduly sensitive to the drug.

The cells from which marrow colonies arise (colony-forming cells) are thought by most workers to be stem cells committed to granulopoiesis (Rickard *et al.*, 1970; Lancet, 1972). The present results show that haemopoietic recovery in this patient was associated with the presence of normal numbers of these cells in a marrow obtained 14 days after the last dose of amodiaquine. The results also show that growth of granulopoietic colony-forming cells from the patient, but not from a normal control, was significantly inhibited by amodiaquine in a concentration comparable with what might have been expected in vivo. Failure of lower concentrations to produce any effect in vitro suggests that the myelosuppression was dose-dependent and not an idiosyncratic reaction. This is also suggested by the fact that symptoms attributable to the consequences of agranulocytosis occurred only after eight doses had been given, although leucocyte counts were not performed before admission and the time of onset cannot be established with certainty.

The technique of culturing human bone marrow on semi-solid agar is well established and easy to perform. We suggest

TABLE II—Results of Bone Marrow Cultures from the Patient and From a Control in the Presence of Increasing Concentrations of Amodiaquine

Feeder Layer	Concentration of Amodiaquine in Patient (µg/ml)				Concentration of Amodiaquine in Control (µg/ml)			
	Nil	0.005	0.05	0.5	Nil	0.005	0.05	0.5
A	42	31	28	10	102	84	104	102
B	60	50	58	16	103	140	139	131
C	35	56	51	25	77	110	103	108
D	76	43	57	22	102	132	135	131
E	71	65	60	18	84	129	144	147
Mean $\pm$ S.D.	57 ± 4	49 ± 4	51 ± 4	$18^* \pm 4$	94 ± 6	$118^* \pm 6$	$125^* \pm 6$	$124^* \pm 6$

*Means which differ significantly ($P < 0.001$) from the mean untreated value for the same marrow sample.

Analysis of variance: patient $F_{3,41} = 26.75$, $P < 0.001$; control $F_{3,41} = 9.61$, $P < 0.001$.

Values are the numbers of colonies grown from plating 2×10^5 nucleated marrow cells. Individual values are the means of triplicate cultures on each feeder layer.

that it be used to investigate other patients with agranulocytosis after exposure to possibly toxic drugs, particularly where more than one drug might be responsible. In the present case cultures were carried out after haematological recovery had occurred. Whether the technique can be used in the acute phase of marrow suppression will depend on the number of colony-forming cells present in the marrow.

Further studies of the patient at a longer interval after recovery, and of her family, are planned, and will be reported subsequently.

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MEDICAL MEMORANDA

Lymphosarcoma Presenting as Löffler's Syndrome

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A case of Löffler's syndrome presenting with respiratory symptoms and a high eosinophilia is reported. Full investigation, including bone marrow biopsy, was carried out with negative results. Four months later further physical signs developed which led to a diagnosis of lymphosarcoma being established by lymph node and bone marrow biopsy. This is a rare finding, and in two extensive reviews of lymphoid tumours Löffler's syndrome was not reported as the presenting clinical picture in lymphosarcoma.

Case Report

A previously healthy 4-year-old boy was admitted to the Royal Manchester Children's Hospital in December 1971. Two weeks before admission he had been treated for a dental abscess with lincomycin. This was discontinued after three days because he developed an urticarial rash. He then had two teeth extracted under general anaesthesia with tetracycline (Achromycin) cover. Two days before admission he was noted to be lethargic and to be sleeping more than usual. Later that day his respiration rate increased and he became flushed. These symptoms persisted until admission, by which time he had developed a dry cough and had vomited three times.

His birth and neonatal history were normal. Both parents and his 8-year-old sibling were well. He had never been abroad, and the family did not have any pets.

On examination he had a temperature of 38.5°C, and his tongue was coated. One small mobile lymph node was palpable in his left inguinal region. There was no other overt lymphadenopathy. He was dyspnoeic with a respiratory rate of 60-70/min but the chest was normal on percussion and auscultation. The cardio-

vascular, alimentary, and central nervous systems were quite normal, in particular the liver and spleen could not be palpated.

On admission radiograph of the chest showed the presence of multiple diffuse nodular opacities throughout both lung fields (see fig.). Other investigations were: haemoglobin 10.2 g/100 ml, total white cell count 35,000/mm³, differential count, eosinophils 71%, neutrophils 11%, lymphocytes 18%.

Chest radiograph showing multiple diffuse nodular opacities throughout lung fields.

His clinical condition rapidly deteriorated with increasing dyspnoea and cyanosis. His liver became palpable, and he complained of epigastric discomfort. The liver was smooth and non-tender with a firm consistency. He was, therefore, nursed in an oxygen tent and given corticosteroid therapy. He improved on this treatment, the epigastric pain eased, and his respiratory symptoms became less severe. The liver gradually diminished in size over a two-week period.

The following tests were carried out shortly after his admission and while he was receiving steroid therapy: E.S.R. 40 mm in the first hour. Liver function tests, the blood urea, serum electrolytes, serum calcium, and serum phosphate were all within normal limits. Cultures of blood, urine, stool, nasal and throat swabs did not show any pathogenic organism. No acid-fast bacilli, worms, or ova were found in gastric washings or in repeated stool specimens. Protein electrophoresis showed an increase in α_2 globulin: serum

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