Protein and energy malnutrition and iron-deficiency anaemia are widespread among preschool children in India. Because of a poor antibody response in severely malnourished children admitted to hospital it has been suggested that mass vaccination programmes would not be effective in populations where malnutrition is widespread. Earlier studies by this institute, however, have shown that most children in the community who suffer from mild or moderate protein and energy malnutrition respond well to conventional immunisation programmes.²⁵ Our present findings show that antibody production is also unaffected by iron deficiency. Thus conventional immunisation programmes may be effective even in undernourished communities.

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SHORT REPORTS

Human basophil degranulation test in diagnosis of hydatidosis

IgE antibodies directed against parasitic antigens in hydatidosis have been measured by the radioallergosorbent test (RAST).^{1,2} After challenge with antigen the basophils bearing specific IgE apparently disappear, and this phenomenon has been used as a diagnostic test in inhalant allergies.³⁻⁵ We report the results of the human basophil degranulation test (HBDT) performed on a suspension enriched with basophils in 12 patients with hydatidosis and in 68 control subjects.

Patients, methods, and results

The controls not suffering from hydatidosis were six newborn babies, 12 adults living in Paris (non-endemic area), and 50 black African adults with parasitic diseases other than hydatidosis. The patients were 10 adults with surgically proved hepatic cyst, one patient with only an epiploic cyst 3 cm in diameter, and one patient with a hepatic calcified cyst and positive serological reaction.

Venous blood 10 ml was drawn from patients and controls on to heparin and centrifuged for 15 min at 150 g to separate the plasma with platelets, which was discarded. The buffy-coat mixed with 5 ml HEPES-buffer⁴ was gently laid on 5 ml gradient density solution (1.080 g/ml, Institut Pasteur) in a round-bottomed plastic tube 100 mm × 18 mm. After centrifugation (400 g at the interface for 30 min) the ring containing lymphocytes and basophils was collected and washed once in HEPES-buffer (150 g, 10 min), which was then discarded except for about 0.5 ml at the bottom of the tube; 20 µl of this enriched cell suspension and 10 µl of the antigen solution (or 10 µl of buffer as control) were deposited in circles of the same surface area on a slide, which was then kept for 15 min at 37°C in a Petri dish containing wet cottonwool. After quick drying the slide was fixed in methanol before being stained with a solution containing toluidine blue (Institut Pasteur); washed successively in distilled water (30 s), ethanol (30 s), and

xylene; and mounted. The basophils were counted in equal numbers of randomly distributed microscopic fields. The degranulation, expressed as the percentage of basophils that apparently disappeared in the presence of antigen, was significant ($p < 0.001$) when higher than 35% if more than 100 basophils were counted in the control circles.⁴ The antigen was the liquid drawn from hepatic cysts during operation before injection of formalin or saline solution. It was distributed in aliquots and kept at -80°C before being used pure and diluted 1:10 and 1:100 in HEPES-buffer.

The basophils almost completely disappeared in 12 patients with pure hydatid liquid. The degranulation was highly significant at that antigen concentration and at lower concentrations except in one patient (case 9) who had an epiploic cyst (table). The degranulation was always below 35% in the 68 controls.

Number of basophils counted in 12 patients with hydatidosis

No of basophils*				
Case No	Without antigen	With antigen		
		Pure†	1:10†	1:100†
1	484	29 (94%)	53 (89%)	126 (74%)
2	392	31 (92%)	71 (82%)	157 (60%)
3	195	52 (73%)	66 (66%)	104 (47%)
4	263	67 (75%)	75 (71%)	63 (76%)
5	147	16 (89%)	31 (79%)	63 (57%)
6	515	16 (97%)	27 (95%)	100 (81%)
7	206	29 (86%)	88 (57%)	ND
8	633	127 (80%)	316 (50%)	297 (47%)
9	404	101 (75%)	331 (18%)	421 (0%)
10	522	68 (87%)	157 (70%)	162 (69%)
11	168	24 (86%)	13 (92%)	32 (81%)
12	188	26 (86%)	55 (71%)	120 (36%)
Mean of degranulation (± SEM)		85 ± 2.2	70 ± 6.1	57 ± 7.3

*Each experiment or control was done in triplicate. Numbers represent the sum of basophils counted in 30 to 60 microscopic fields (10 to 20 fields per circle).

†Between parenthesis: % degranulation.

Comment

The technique of human basophil degranulation has not been used for diagnostic purposes because these cells are few in number. Nevertheless we^{4,5} and others³ have shown their usefulness when obtained in large amounts through partial enrichment. Our results in hydatidosis show that HBDT is a very sensitive test, since the degranulation was highly significant for at least one antigen dilution in all patients. Moreover, HBDT proved to be specific—we never had a false-positive result, even in controls suffering from another helminthiasis. Finally, the results were reproducible, as proved in two patients retested after an interval of a few days.

We conclude that HBDT is a useful diagnostic test for hydatidosis, and its simple technology could be made available for endemic areas. We are studying its value in diagnosing schistosomiasis.

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Malaria with disseminated intravascular coagulation and peripheral tissue necrosis successfully treated with streptokinase

Disseminated intravascular coagulation sometimes complicates malaria^{1,2} but there is no report of peripheral ischaemia resulting from it. We describe two white children (brothers) infected at the same time and developing disseminated intravascular coagulation with tissue ischaemia, one of whom was treated with streptokinase.

Case reports

Both brothers gave a three-day history of vomiting, rigor, and faintness. Both had stopped malarial prophylaxis in error and both showed many ring forms of *Plasmodium falciparum*, which persisted for three days after they started treatment.

Case 1 The 11-year-old brother was relatively well on admission, though vomiting and dehydrated. He was rehydrated with normal saline and treated with chloroquine, equivalent to 150 mg base twice daily. Two days later he had become somewhat drowsy and he had a spreading area of necrosis on the dorsum of the right hand starting at a venepuncture site and extending into the ring and small fingers. Results of investigations were: plasma urea 10.46 mmol/l (63 mg/100 ml); sodium 131 mmol (mEq)/l; potassium 5 mmol (mEq)/l; fibrinogen 1.4 g/l; thrombin time 8 seconds; fibrinogen degradation products (FDP) 4.0 g/l; haemoglobin 11.6 g/dl; platelets $21 \times 10^9/l$ ($21\,000/mm^3$); schistocytes present. Disseminated intravascular coagu-

lation with impending tissue necrosis was diagnosed, and treatment was started with heparin 2500 units four-hourly, with a single dose of quinine 300 mg in saline infused over two hours. Two days later the patient was afebrile but his haemoglobin was 10.4 g/dl, platelets were $36 \times 10^9/l$ ($36\,000/mm^3$), and fibrinogen was 0.5 g/l. The platelet and fibrinogen values did not return to normal until four days later.

Case 2 The 9-year-old brother of the first patient was in a considerably worse condition on admission. He was very drowsy and feverish. Cerebral malaria was provisionally diagnosed and he was given dexamethasone 2.5 mg eight-hourly, a single dose of quinine 300 mg in saline over two hours, and chloroquine. His condition worsened over 24 hours. He became very confused and his right foot grossly ischaemic with a demarcation line above the ankle (fig). Results of investigations were: haemoglobin 12.9 g/dl; platelets $10 \times 10^9/l$ ($10\,000/mm^3$); fibrinogen 1.8 g/l; thrombin time 9 seconds; FDP 4.0 g/l. Heparin 2500 units four-hourly brought no improvement. Indeed, 36 hours later the patient could barely be roused, the toes of his left foot became dusky blue, and the right foot was worse. Pulses were palpable in both feet. In view of the gross ischaemia and failure to respond, heparin was discontinued and replaced with streptokinase, 200 000 units initially and then 50 000 units hourly, preceded by two units of fresh whole blood and three units of platelet concentrate. Six hours later there was a noticeable improvement in the perfusion of his feet. The haemoglobin was 14.2 g/dl; platelets $21 \times 10^9/l$ ($21\,000/mm^3$); FDP 1.0 g/l; fibrinogen 0.3 g/l; thrombin time—clot formed at 60 seconds and lysed at 140 seconds. Because of the very low fibrinogen cryoprecipitate 4 units was given. In a further 24 hours the foot was clearly viable and had begun to blister and the patient was conscious. The haemoglobin was 12.8 g/dl; platelets $78 \times 10^9/l$ ($71\,000/mm^3$); FDP 1.0 g/l; thrombin time—clot formed in 7 seconds and lysed in 10 seconds. The streptokinase was discontinued and heparin restarted. In a further 24 hours platelets and fibrinogen were normal.

Both children completely recovered.

Feet of 9-year-old boy with disseminated intravascular coagulation and peripheral ischaemia associated with malaria. Solid lines (a) show the demarcation between normal and cyanotic tissue; broken lines (b) show areas of skin returned to normal colour six hours after start of streptokinase treatment.

Comment

Extensive tissue necrosis resembling purpura fulminans has not previously been described as a complication of malaria. Its occurrence at the same time in two brothers raises the question of whether disseminated intravascular coagulation can be an inherited reaction pattern. Alternatively, a particularly virulent form of malaria may be implicated since the two boys were presumably infected by bites from the same mosquito. The disseminated intravascular coagulation in both cases was prolonged well after adequate treatment for malaria had begun. The use of streptokinase has been previously described in purpura fulminans,³ and in view of the failure of heparin and the rapid improvement of the ischaemia with streptokinase it seems to have been of value in the present case. Its use, however, when bleeding is a complication is clearly not without con-