although this effect is hardly demonstrable in vitro (prostacyclin is so labile that it has disappeared by the time P.R.P. is prepared for in-vitro aggregation studies). We therefore decided to study whether Bay g 6575 stimulates the release of prostacyclin by the vessel wall.

Bay g 6575 is barely soluble in aqueous solvents. Since organic solvents may damage the rings of aorta, we used instead plasma obtained before and after ingestion of the drug. The experiments showed that plasma obtained after drug ingestion stimulates the release of a platelet-aggregation inhibiting substance from the arterial wall. Since the inhibiting substance is not released when the vessel rings are pretreated with acetylsalicylic acid, which is a specific inhibitor of arachidonic acid transformation into prostacyclin, it is reasonable to conclude that this substance is prostacyclin. Further experiments indicated that the drug stimulates prostacyclin release from the vessel wall rather than stabilising released prostacyclin. Plasma obtained after ingestion of Bay g 6575 is very similar to uræmic plasma, which, as recently demonstrated by Remuzzi et al.,[13] also stimulates prostacyclin release from the vessel wall.

The present study used exhausted rat-aorta rings. Normal vessel wall possesses a strong prostacyclin-degrading system.[14] It follows that in fresh vessel rings, which release prostacyclin into the surrounding medium, synthesis of prostacyclin must exceed its degradation. In exhausted rings, however, degradation would exceed synthesis. The stimulation of prostacyclin release from exhausted vessels by Bay g 6575 would thus be due to a resurgence of prostacyclin generation, or, more probably, to inhibition of prostacyclin degradation; further experiments are required to distinguish these possibilities.

Stimulation of release of prostacyclin from the vessel wall by Bay g 6575 may be the way by which this drug reduces the weight of 24 h old thrombi in animals. Indeed, prostacyclin not only prevents aggregation but also disrupts already existing aggregates.[15]

It has recently been shown that dipyridamole acts as an antithrombotic agent by potentiating endogenous prostacyclin.[16] In order to confirm that Bay g 6575 stimulates the release of prostacyclin from the vessel wall in vivo in man, we studied whether the effect of the drug was potentiated by dipyridamole. We found that although Bay g 6575 alone or dipyridamole alone had no clear effect on platelet aggregation in vitro, their combined administration led to a very striking and prolonged inhibition.

In conclusion, we believe that the antithrombotic properties of Bay g 6575 are the result of stimulation of prostacyclin release from the vessel wall, and that this effect is also demonstrable in humans. Since the drug is active orally and, from present evidence, is well tolerated, it deserves detailed assessment as an antithrombotic agent in man.

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TRANSMISSION OF NON-A NON-B HEPATITIS TO CHIMPANZEES BY FACTOR-IX CONCENTRATES AFTER FATAL COMPLICATIONS IN PATIENTS WITH CHRONIC LIVER DISEASE

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Summary 6 cases of non-A non-B hepatitis which followed administration of four different batches of concentrates of coagulation factor IX from commercial and non-commercial sources are described. Of 17 patients who received the concentrate on account of chronic liver disease, 4 developed hepatitis, and in 3 of these the illness proved fatal. The incubation periods ranged from 42 to 103 days (mean 65 days). 3 chimpanzees were inoculated with concentrate from the same batch used on the above patients, a further commercial batch upon which no adverse reactions had been reported, and plasma from a known non-A non-B carrier. All developed hepatitis after 10 weeks' incubation. Liver biopsy when serum-aminotransferase was at its highest level showed features consistent with acute hepatitis. As in the patients, viral markers for hepatitis A and B, cytomegalovirus, and Epstein-Barr virus were unchanged.

Introduction

SCREENING of donor blood and blood products for hepatitis-B surface antigen has led to a reduction, but not complete elimination, of post-transfusion hepatitis. When hepatitis A and B and the other viruses, cytomegalovirus and Epstein-Barr, that may involve the liver have been excluded, there remain a number of cases attributable to what has been called non-A non-B hepatitis.[1,2] Examination of acute and convalescent sera from such cases has shown the existence of a possible antigen/antibody system,[3] and direct evidence of a transmissible agent comes from the transmission of non-A

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non-B hepatitis by blood from implicated donors and patients to chimpanzees.[4,5]

In this paper we describe the occurrence in 6 patients of non-A non-B acute hepatitis following administration of concentrates of coagulation factor IX. In 4 patients with underlying chronic liver disease the concentrate was given before liver biopsy and formed part of a prospective study of the value of such replacement. In the other 2 patients another concentrate of factor IX had been given for rapid correction of oral anticoagulation. The infectivity of these preparations was shown by successful transmission of hepatitis to chimpanzees.

Patients and Schedule of Concentrate Use

The 4 patients with liver disease were jaundiced and had a prolonged prothrombin-time of 6–11 s despite parenteral vitamin K. Liver biopsy was carried out in each instance because of doubt about the underlying cause of the possible cirrhosis. Patients 1 and 3 had a history of high alcohol intake, and patient 2 had biochemical and clinical features suggestive of Wilson's disease. After administration of the concentrate, patients were reviewed every 6 weeks, with estimation of liver function and serum markers of hepatitis A and B virus infection.

The remaining 2 patients had chronic valvular heart-disease and were on long-term oral anticoagulant therapy as prophylaxis against embolic complications. Both, for various reasons, had previously undergone liver biopsy which had shown minor hepatic fibrosis and hepatocellular fatty change. In patient 5 the concentrate was given before a minor surgical procedure and in patient 6 after excessive anticoagulation.

Patients 1–4 received 1000–2000 units of a commercial factor IX, and patient 2 also received 500 units of factor-VII concentrate. In patient 1 a different batch of concentrate from the same manufacturer was used, and each of the 2 patients on oral anticoagulants received 1300 units of different batches of a factor-IX concentrate from another manufacturer. In all patients the concentrate was dissolved in 30 ml of water for injection and administered as an intravenous bolus.

Results

After administration of the concentrate in patients 1–4, the prolonged prothrombin-time was reduced to within 4 s of the control; and adequate liver-biopsy specimens were obtained uneventfully. On the basis of the histological appearances and other findings, patients 1 and 3 were finally considered to have alcoholic cirrhosis, patient 2 Wilson's disease, and patient 4 cryptogenic cirrhosis.

All 4 patients were discharged home and remained clinically stable until 42–100 days after concentrate infusion. The 2 patients receiving concentrate for oral-anticoagulant reversal also remained well until this time. In each instance anorexia, malaise, and jaundice developed, and serum-aspartate-aminotransferase (A.S.T.) rose sharply—in 4 cases to 1000–2700 I.U./l (see table). These changes were accompanied by a rise in serum-bilirubin of up to 850 I.U./l and prolongation of the prothrombin-time by up to 107 s.

The most rapid and severe deterioration of liver function was seen in patients 1–3, in whom the illness finally proved fatal. Patient 1 became encephalopathic within a week of malaise developing, and during the first 24 h after admission the level of consciousness deteriorated further and he became deeply jaundiced. The liver-function tests were severely deranged, with serum-A.S.T. elevated to 2700 I.U./l. The prothrombin-time was prolonged by 107 s (see table). He died 2 weeks after the apparent onset of the hepatitis and 5 days after the development of coma. Patient 2, who had Wilson's disease, had been started on penicillamine treatment shortly after the biopsy. When reviewed 10 weeks later his condition was stable and there was no ascites. 3 weeks later he developed nausea, vomiting, and jaundice, which increased during the following 2 weeks. On the day before readmission he showed signs of encephalopathy. Liver-function tests showed elevation of serum-A.S.T. to 2150

CLINICAL AND BIOCHEMICAL DATA (PEAK ABNORMALITY RECORDED) OF 6 PATIENTS

Patient no.	Age	Sex	Underlying liver disease	Factor-IX concentrate	Interval between concentrate and hepatitis (days)	Liver-function tests before concentrate and during hepatitis							
						Prothrombin-time (s prolonged)		Bilirubin (μmol/l)		Alkaline phosphatase (I.U./l)		A.S.T. (I.U./l)	
						Basal	Peak	Basal	Peak	Basal	Peak	Basal	Peak
1	53	M	Alcoholic cirrhosis	Batch A, 1000 units	42	7	107	29	580	158	157	100	2700
2	14	M	Wilson's disease +cirrhosis	Batch B, 1000 units	103	11	90	74	174	700	950	540	2150
3	56	F	Alcoholic cirrhosis	Batch B, 2000 units	65	7	13	110	850	264	340	110	1300
4	57	M	Cryptogenic cirrhosis	Batch B, 2000 units	56	7	14	40	136	151	286	100	1280
5	68	M	Fibrotic +fatty changes	Batch C, 1300 units	70	..	..	13	117	78	200	86	550
6	61	F	Fibrotic +fatty changes	Batch D, 1300 units	55	..	..	13	197	102	287	68	900

GRO-A 9D1057 GRO-A

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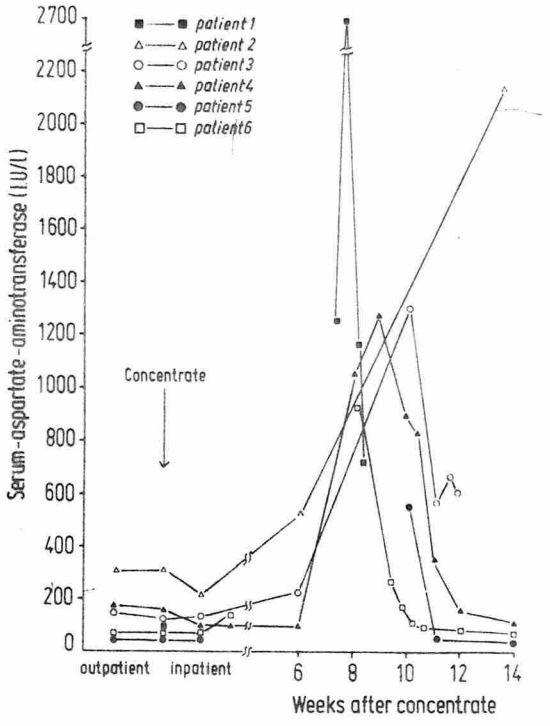

Fig. 1—Serial serum-aspartate-aminotransferase values for 6 patients with hepatitis, following administration of factor-ix concentrates.

I.U./l (fig. 1), and prolongation of the prothrombin-time by 90 s (see table). He died the next day. Patient 3 had become increasingly jaundiced, with an increase in ascites and mental confusion, 65 days after concentrate therapy. On admission the liver-function tests showed a similar pattern of changes to the previous cases (see table); and during the next week the level of consciousness progressively deteriorated, and the serum-bilirubin rose to 350 μmol/l (fig. 1). She died 2 weeks after admission, 83 days after concentrate therapy.

The 1 patient with underlying liver disease who survived (patient 4), a man with previously well-compensated cryptogenic cirrhosis, returned 56 days after the concentrate infusion, having noticed malaise and, for the first time, ascites. The serum-A.S.T. was elevated to 1280 I.U./l. Over the next 6 weeks, with clinical recovery, serum-A.S.T. returned to the level at presentation (fig. 1).

In patients 5 and 6, who had no pre-existing major liver disease, the onset of malaise and jaundice occurred at a similar time. Biochemical changes were less severe than in patients 1–4, although the levels took up to 2 months to return to the values found before administration of the concentrate (table and fig. 1).

Liver biopsy was performed in 5 of the 6 patients (except patient 5, in whom long-term oral anticoagulant therapy had been restarted), either at the time of maximum elevation of aminotransferase or immediately after death; in each instance features of an acute hepatitis were found. In patients 1–4 these were superimposed on changes of chronic liver disease and were characterised by spotty necrosis, acidophilic bodies, and heavy inflammatory-cell infiltrate. Comparison of these biopsy specimens with those obtained at the time of administration of concentrate showed a striking increase of inflammatory-cell infiltrate and a change in the predominant cell type from a polymorphonuclear to a mixed cell infiltrate with prominent plasma-cells (figs. 2 and 3). In the 3 patients who died the liver showed a considerable reduction in hepatic parenchyma compared with the initial biopsy.

In all 6 patients examination of sera during the peak elevation of aminotransferase or on readmission was negative for HB_sAg. Only in patient 4 was antibody to HB_sAg detected; and this had been present in serum taken before concentrate therapy. Similarly, antibody to hepatitis-A virus was detected in serum from patients 3, 4, and 6 but was also present before concentrate therapy in 2 of them, no previous serum sample being available for patient 6.

Since there is one report of an association between transfusion of blood containing an elevated level of carcinoembryonic antigen (C.E.A.) and the development of

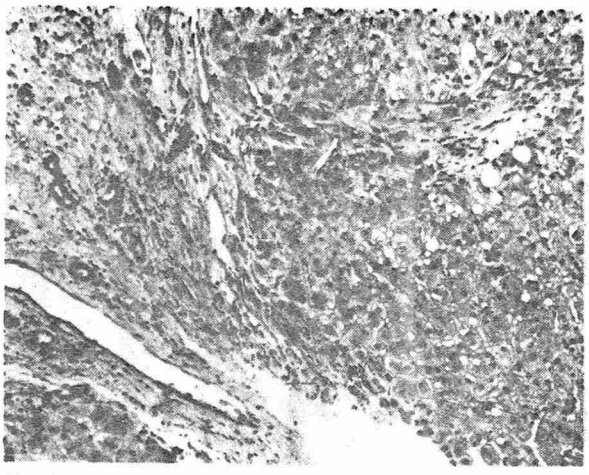

Fig. 2—Liver-biopsy material from patient 4, showing features of cryptogenic cirrhosis with minimal cellular infiltrate confined to fibrous portal areas.

Hæmatoxylin and eosin, reduced by ¼ from ×120.

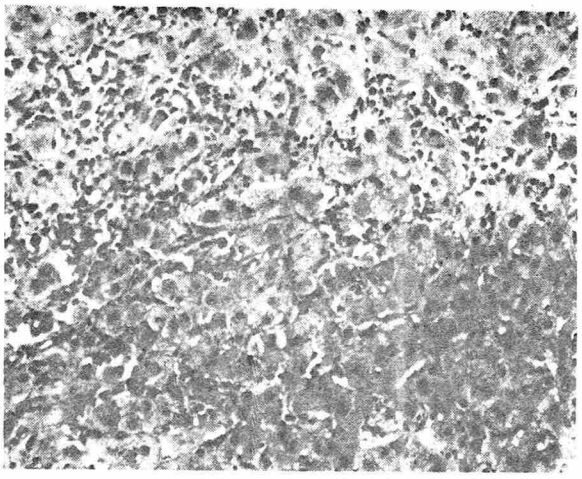

Fig. 3—Liver-biopsy material from patient 4 during peak elevation of serum-aminotransferase, showing portion of regenerating nodule with spotty necrosis and severe cellular infiltration throughout.

Hæmatoxylin and eosin, reduced by ¼ from ×160.

post-transfusion hepatitis,[6] C.E.A. levels were measured by radioimmunoassay. In the concentrate batch B levels were within the normal range (up to 20 ng/ml), and although serum-levels taken during hepatitis were elevated in patients 3 and 4 (56 and 69 ng/ml respectively), they were not statistically different from levels found before concentrate therapy.

Transmission of Hepatitis to Chimpanzees

The three young male chimpanzees used during the experiment were kept at the primate unit of the London School of Hygiene and Tropical Medicine. Chimpanzee George had previously been experimentally infected with hepatitis viruses A and B; chimpanzees Jeremy and Victor had not been used in experiments and were negative for hepatitis-B virus serological markers but had circulating antibody to hepatitis-A virus. Inoculation with the test material followed a 4-week period of baseline observations during which twice-weekly liver-function tests and at least two liver biopsies were obtained. All had normal hepatic histology before inoculation.

Chimpanzee Jeremy was inoculated intravenously with 1500 units of batch B of the factor-IX concentrate, implicated in cases 2, 3, and 4, dissolved in 30 ml of water. After 10 weeks serum-alanine-aminotransferase (A.L.T.) had risen from 20 to 60 Karmen units/ml (upper limit of normal 20 Karmen units/ml) (fig. 4). Serum-A.L.T. fell again during the 12th week and returned to normal by the 15th week after inoculation. Chimpanzee George was inoculated with the same dose of a batch E of factor-IX concentrate, not administered to the present patients but used in Europe apparently without complication. In the 10th week after inoculation serum-A.L.T. rose to 30 Karmen units/ml and during the next 2 weeks rose further (fig. 4), returning to normal by week 14. Chimpanzee Victor acted as a positive control, being inoculated with 2 ml of plasma from a known non-A non-B hepatitis carrier (provided by Dr H. J. Alter and Dr R. H. Purcell) and previously successfully transmitted to a chimpanzee in America. After the same 10-week incubation period serum-A.L.T. had risen to 90 Karmen units/ml and by the 12th week to 120 Karmen units/ml (fig. 4).

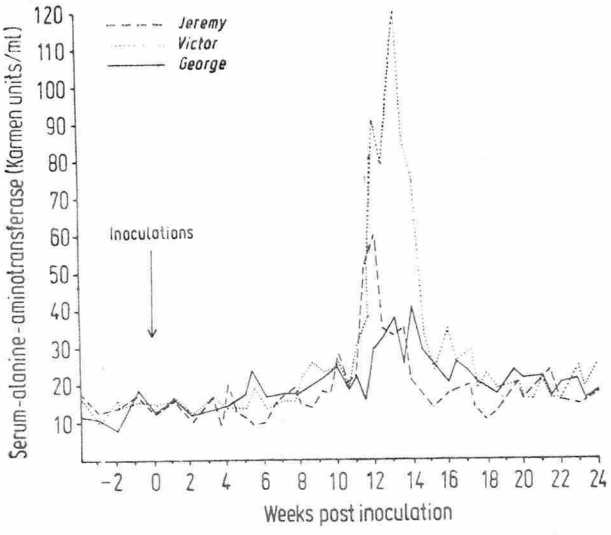

Fig. 4—Serial serum-alanine-aminotransferase values in three inoculated chimpanzees.

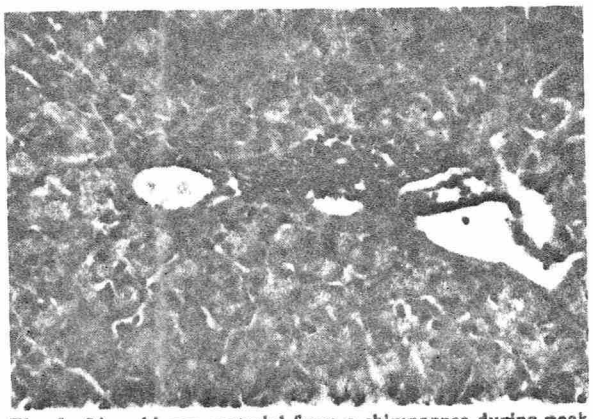

Fig. 5—Liver-biopsy material from a chimpanzee during peak elevation of aminotransferase, showing inflammatory-cell infiltrate of portal tract and prominence of Kupffer cells.

Hæmatoxylin and eosin, reduced by 2/5 from ×260.

Percutaneous liver biopsies at the peak aminotransferase level showed evidence of acute viral hepatitis—periportal and intralobular accumulation of inflammatory-cell infiltrates (fig. 5) with intralobular focal areas of necrosis. The hepatocytes showed mild cytoplasmic vacuolation. Prominence of the Kupffer cells was a constant and striking feature. The histological changes in the biopsy specimens obtained from chimpanzee George were less striking but consistent with acute hepatitis. Low-power electron microscopy confirmed the acute cytopathological changes. Sequential liver biopsies from chimpanzee Jeremy have shown some evidence of progression to chronic liver damage.

Serological markers of hepatitis A and B virus infection, cytomegalovirus, and Epstein-Barr virus remained unchanged.

Discussion

All 6 patients had a definite episode of hepatitis, as shown by the clinical symptoms and biochemically by development of jaundice, sharp rise in aminotransferase (in 4 patients to more than 1000 I.U./l), and prolongation of the prothrombin-time. All these features appeared at a similar interval after the concentrate therapy. Until that time the condition of the 4 patients with chronic liver disease had been stable. In the three fatal cases the pattern of illness resembled that seen in patients with fulminant viral hepatitis, and no other cause was found for the sudden deterioration. In particular, there was no evidence of intercurrent infection or gastrointestinal hæmorrhage. Other known causes of post-transfusion hepatitis—i.e., hepatitis viruses A and B and cytomegalovirus—were also excluded.

Although the features of non-A non-B hepatitis are, in general, similar to those of hepatitis B, it is a milder illness, and only 20% of patients became jaundiced. Its fulminant course in 3 of our patients could have been due at least in part to their underlying chronic liver disease, making them more susceptible to the parenterally administered agent.

That the hepatitis was non-A non-B and was likely to have been transmitted by the factor-IX concentrate is further shown by definite biochemical and histological

features of a hepatitis observed in the chimpanzee Jeremy after inoculation with batch B. These features were comparable to those seen in other reported studies on non-A non-B hepatitis transmission. The incubation period of 10 weeks agrees well with the 13·4 weeks reported by Alter et al.[4] but is longer than the 2–4 weeks observed by Tabor et al.[5] in transmission experiments. The variation in incubation period in the chimpanzee contrasts with that found in man (mean incubation period in this and the other two series[4,5] is 7–8 weeks). Such differences may be due to variations in host response or possibly the titre of virus in the inoculum, and there is also evidence in man for more than one non-A non-B hepatitis agent.[7] As in other studies of non-A non-B hepatitis transmission in chimpanzees, serum-A.L.T. proved to be more valuable a marker of liver damage than serum-A.S.T.

Four different batches of factor-IX concentrates from two manufacturers were involved in these cases, and the occurrence of hepatitis in chimpanzee George after administration of batch E means that a fifth was also potentially infective. These factor-IX concentrates are in wide use in many centres, but there are at least two possible reasons why hepatitis has not previously been reported. Firstly, non-A non-B hepatitis is usually a subclinical anicteric illness and can be easily overlooked in patients sick from other illnesses.[8,9] Secondly, patients with congenital disorders of hæmostasis, in whom most of the experience with coagulation concentrates has been obtained, may have acquired immunity from previous non-A non-B hepatitis either passively or from previously administered blood-products.

Until blood-donors can be screened for the non-A non-B hepatitis agent, it would seem wise to restrict the use of both commercial and non-commercial concentrates to life-threatening situations. In particular, their use in patients with chronic liver disease should be avoided, as the risk of a serious illness resulting appears to be increased.

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SEVERE MALARIA AND GLUCOSE-6-PHOSPHATE-DEHYDROGENASE DEFICIENCY: A REAPPRAISAL OF THE MALARIA/G-6-P.D. HYPOTHESIS

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Summary Nigerian children with convulsions and *Plasmodium falciparium* parasitæmia above 100 000/μl did not show a decreased frequency of glucose-6-phosphate-dehydrogenase (G.-6-P.D.) deficiency. A re-evaluation of earlier studies has led to the conclusion that clinical evidence of protection against falciparum malaria in G.-6-P.D.-deficient individuals is lacking. Evidence for the possible role of malaria in selecting for G.-6-P.D.-deficient genes consists solely of the geographical association of high frequencies of G.-6-P.D. deficiency with endemic malaria.

Introduction

Allison observed that the geographical distribution of some red-cell polymorphisms corresponded to areas of the world were malignant falciparum malaria was endemic and suggested that the genes involved in these polymorphic systems carried a selective advantage in these areas.[1,2] Protection of the sickle-cell heterozygote against malaria has been confirmed repeatedly in clinical studies[3,4] and in vitro.[5,6] Clinical evidence for an association between glucose-6-phosphate-dehydrogenase (G.-6-P.D.) deficiency and reduced susceptibility to disease is less convincing.[7] In a study of G.-6-P.D. polymorphisms in Nigerian children with severe malaria and convulsions we have failed to find any evidence of protection of G.-6-P.D.-deficient children.

Subjects and Methods

90 Nigerian children (predominantly Yoruba) with convulsions who were brought to St. Mary's Hospital, Eleta, Ibadan, Nigeria, between June and September, 1977, were studied. None had received medication except traditional remedies. Blood was obtained on admission, and parasitæmia was determined on Giemsa-stained thin films by counting the number of infected erythrocytes per 500 erythrocytes. Parasitised red cells/μl were estimated from the percent parasitised red cells and hæmoglobin concentration. Hæmoglobin and G.-6-P.D. electrophoresis were done on starch gel,[8] and G.-6-P.D. activity was determined by spectrophotometric assay.[9] Any sample with enzyme activity <40 units/g Hb was considered deficient. This corresponds to genotypes Gd^{A-} for males and Gd^{A-}/Gd^{A-} for females. Such samples invariably ran as the faster-moving electrophoretic band, and the band stained faintly. It was im-