In January, 1972, patients with cutaneous hemorrhages and sometimes with mucosal hemorrhages were observed among new settlers in the Altamira region of Brazil. Thrombocytopenic purpura was initially diagnosed. A multidisciplinary team from the Instituto Evandro Chagas, Belém, Brazil, began studies to characterise the clinical, laboratory, and epidemiological aspects of this disease and discover its cause. This is the first report on the disorder, which we have called the hemorrhagic syndrome of Altamira (H.S.A.).

Clinical Features

Of 92 patients observed, 76 were seen in Altamira and 16 were in hospital in Belém, where they were followed for 1 to 3 months. The 16 patients in Belém were observed by us and standard forms were used to collect clinical information for those in Altamira.

Findings.—The disease usually started with the appearance of hemorrhages into the skin followed sometimes by bleeding of the mucous membranes. In one patient the latter sign was noted first. The hemorrhages into the skin took the form of multiple petechiae and ecchymoses, covering the whole surface of the body or restricted to the face and extremities. Ecchymoses 5 mm. in diameter were recorded. Spontaneous bleeding from skin lesions caused by insect biting as well as traumatic hematomas in the subcutaneous tissue were observed. Bleeding from the mucous membranes occurred in about 30% of the cases (28/86), the chief sites being the gums and nasal mucosa. Petechiae were observed in the mucosa of the mouth. Melena and menorrhagia were rarely seen. Hematuria was not detected by microscopic examination.

Most patients were anemic (49/85). Most did not have fever, which when present was usually low grade. A few individuals (3/78) did have temperatures of 38°C and several others reported a mild febrile period (9/17) preceding the bleeding-signs. Although several patients complained of asthenia, and were severely ill, there were no signs of altered cerebral function. Diarrhoea, vomiting, jaundice, myalgias, tremors, paresthesias, and convulsions were not encountered. Lymphatic nodules, usually palpable in groins and axilla, were small and not painful. Mild hepatomegaly, splenomegaly, and oedema of the legs were seen only occasionally.

Treatment.—Initially patients with severe bleeding received blood transfusions and milder cases were given intravenous glucose. Broad-spectrum antibiotics, iron, and vitamins were given to most of them, but limited improvement followed this therapy. Improvement was remarkable, however, when daily doses of 20–40 mg. of prednisone were administered. In 5

The Lancet · Saturday 13 April 1974

Hæmorrhagic Syndrome of Altamira

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Introduction

THROMBOCYTOPENIC purpura is easily diagnosed by clinical and hematological findings. Cases are classified as primary (of unknown origin) or secondary (of known origin). Secondary thrombocytopenic purpura is associated with several etiological factors and clinical conditions such as: the toxic action of drugs and chemicals on the blood and bone-marrows, clinical disorders such as leukemias, aplastic anemia, and disseminated lupus erythematosus; certain viral infections such as dengue hemorrhagic fever and Argentinian hemorrhagic fever; and insect bites and the venom of certain snakes.

Summary

Since January, 1972, a hemorrhagic syndrome has been observed among immigrants living in forested areas along the newly opened Transamazon Highway in Brazil. Most cases are found around Altamira. The illness is characterised by localised and disseminated cutaneous hemorrhages, which are associated with mucosal bleeding in 32% of patients. Platelet-counts are reduced, and there is prolonged bleeding-time and poor clot retraction in about half of the cases. The illness was diagnosed as thrombocytopenic purpura.

Familial clustering of cases was found, with more children being involved than adults. A few deaths have been attributed to the disease. The precise etiology of this condition remains obscure. Drugs, alimentary factors, and various viral, rickettsial, and bacterial pathogens do not seem to be involved. Epidemiological and serological evidence suggests that the syndrome is associated with the black-fly, Simulium, and its bite. Case-rates in the Altamira region began to rise shortly after the appearance of large numbers of simulids in the rainy season, and fell with the approach of the dry season and virtual disappearance of black-flies. Colonists in this area reported intense biting during the rainy season. The syndrome is not seen among natives of the region, most of whom live in the city of Altamira, where simulids are much less common. Patients responded rapidly to steroid therapy, with platelet-counts returning to normal within a few days. The disorder has been named the hemorrhagic syndrome of Altamira.
patients, platelet-counts were done before and after treatment. Although thrombocytopenia was present twenty to sixty days after onset of the disease and remained after supportive therapy, platelet levels returned to normal within two to six days of initiating steroid therapy. The same dramatic recovery has been seen in all other individuals given this therapy. Use of steroids was discontinued upon disappearance of symptoms, and the patients were returned to their homes. H.S.A. recurred in 6 out of 45 persons after periods varying from a month to a year. Whether these represent true relapses is not known. For the past year prednisone has been recommended to practitioners in the area for treatment of H.S.A., with good results.

**Epidemiology**

**Setting.**—The area where cases occurred is a virgin tropical rain forest (coordinates approximately 4° S by 53° W at an altitude of 60 m.) located 20 to 50 miles from the city of Altamira (fig. 1). There is a rainy season from December to May and a dry season from June to November. Over a period of thirty-six years temperatures in Altamira ranged from 20.7 to 31.9°C, and rainfall ranged from 176 to 346 mm. per month in the rainy season and from 26 to 77 mm. per month in the dry season.

**Background.**—Immigration into the area was started in the middle of 1971 as part of a Government operation to colonise the forested areas along the newly opened Transamazon Highway. Immigrants are recruited from many parts of the country, but most are from the states of the north-east. At the highway they live in scattered houses, in small communities called "agrovillas" which contain 48–64 houses, or in a larger settlement called an "agropolis". They practise cut-and-burn agriculture, planting corn, rice, and beans as their main crops.

Most diseases found among the settlers are the same as those seen among native residents, such as malaria and intestinal parasites. Some cases of schistosomiasis have been detected among the immigrants, though the disease does not appear to be common and no local transmission has been observed.

**Outbreaks.**—The first cases of thrombocytopenic purpura in the area were observed in January, 1972. Old residents say that sporadic cases of the disease have been seen for many years, but only among newly arrived settlers. This accords with our observations. 22 cases were observed in 1972, in a population of approximately 7300 colonists, whereas from January to August, 1973, there were 70 cases in a total of about 15,000. On the basis of clinical and laboratory data, the disease was responsible for 3 deaths. Another 4 fatal cases were reported, but were not available for investigation. The disproportionate increase of cases in relation to the increase in susceptible population levels is at least partially due to increased efficacy of surveillance in the second year of the study. In 1972, the number of cases reached a peak in February, then declined to zero by May. Cases reappeared in January, 1973. There was a peak in February and another in May, with a trough in March (fig. 2). Peaks in both years coincided with the rainy season. The disease has not been seen in the dry season.

Of a total of 92 cases, 30 belonged to nine families. The first 9 patients studied were brothers and sisters...
in a family which had come from the north-east. About a month after arrival, 2 of their members started to have haemorrhages. In the next six weeks 7 other siblings experienced the same signs. Only the parents and 2 other children did not have signs of haemorrhage in 1972. In 1973, however, the father had a few skin ecchymoses and his platelet-count dropped to below 100,000 c.mm. A similar sequence of events has been seen among many of the other newly arrived immigrants.

The ages of those cases occurring in 1972 ranged from eight months to fifty years, but cases were ten times more common among those under eighteen than among the older age-group. Although there were slightly more female patients than male patients, the sex composition of the population is not known.

Etiological factors.—Patients were questioned and studies were made to determine whether some local factor could be responsible for the disease. Neither patients nor their contacts could remember taking any of the drugs known to be associated with thrombocytopenic purpura or having any history of food intoxication. Urticarous caterpillars were sought around the houses of two of the families with high percentages of sick members. The only caterpillars or pupae seen were those of common species known to be harmless to man. Hematophagous arthropods are present in no greater numbers than in other areas where the disease is not seen. However, black-flies of the genus Simulium are abundant in the rainy season and bite people with a tremendous ferocity during the day. One of the settlers had built a tower next to his home in an attempt to get himself and his family away from the flies—unfortunately, he could not build it high enough.

Two species of Simulium were found during this study. One has been tentatively identified as S. amazonicum, the other is as yet unidentified. Human-bait studies were started in November, 1972 (fig. 2). Captures were made three times during the week from 7 to 8 A.M., and from 4 to 5 P.M. Two peaks were seen; one in January and one in April, with a trough in February. During the dry season the flies are almost absent.

Laboratory Examinations

Clinical.—Hematological tests were performed by standard methods. Most platelet-counts recorded were under 100,000 c.mm., some of them were as low as 10,000 c.mm. 61% (55/89) of the patients examined had a prolonged bleeding-time, sometimes over thirty minutes. Clot retraction was poor or absent in 51% of the patients (41/80). Capillary fragility (determined by the tourniquet test) was less conspicuous. Coagulation-time, prothrombin levels, and fibrinogen levels were normal. Serum-glutamic-oxaloacetic-transaminase, serum-glutamic-pyruvic-transaminase, and blood-urea were normal or moderately increased, and urinalysis showed no abnormalities. Anemia (males less than 4,500,000 and females less than 3,500,000 red blood-cells per c.mm.) was found, but most patients had hookworms and iron deficiency. The white blood-cells did not vary significantly in number or proportion. Bone-marrow biopsy specimens were taken from two patients. Megakaryocytes were increased, but were not forming platelets. Histopathology of the organs of one fatal case revealed splenic lesions compatible with thrombocytopenic purpura.

Isolation of agents.—Attempts were made to isolate viruses and bacteria from materials collected from patients in the early and late phase of the disease. Two-day-old and three to four week-old Swiss mice were intracerebrally inoculated with 0·02 ml. specimens and adult hamsters were inoculated with 0·02–0·05 ml. intraperitoneally. With the exception of a few non-specific deaths, all animals survived the three-week period of observation. Specimens were also inoculated into VERO and H.Ep.2 cell-cultures and observed for two–three weeks for the presence of cytopathic effect. One strain of herpes-simplex virus was isolated in H.Ep.2 cells inoculated with the throat swab from a patient. Blood-cultures were made in tryptose-phosphate broth (T.P.B.), but all were negative. In 1973, blood from an additional 10 patients was cultured in T.P.B., and in Fletcher’s medium supplemented with 5% inactivated rabbit blood and 0·5% haemoglobin. These cultures were also negative. Cultures from the urine, faces, and throat swabs were performed in standard methods. All the media used were made by Difco. With the exception of Staphylococcus aureus from one fecal sample and an enteropathogenic Escherichia coli from another, only non-pathogenic bacteria were isolated.

No viral agent was isolated from 732 pools prepared with 35,328 Simulium and inoculated intracerebrally into baby mice. The flies had been collected near the house of a family with several cases of the disease. Several litters of mice died. An Enterobacter sp. was cultured from their brains, despite the fact that the insect suspensions were treated with penicillin and streptomycin and then centrifuged before inoculation.

From August to September, 1971, 402 wild marsupials and rodents were captured. Blood and viscera from the animals were inoculated into baby mice. No viruses were isolated. Exposure of sentinel monkeys and chickens was started in November, 1972. Weekly platelet-counts on these animals were always normal. Attempts to isolate viruses from blood specimens have been negative. Furthermore, no viruses have been found in 306 pools made from 3918 mosquitoes collected in the area from November, 1972, to August, 1973.

Serology.—Serology performed in our laboratory and at the Walter Reed Army Institute of Research.
RESULTS OF TESTS FOR HUMAN PRECIPITATING ANTIBODIES TO BLACK-FLY EXTRACTS BY OUCHTERLONY DOUBLE-DIFFUSION-IN-GEL TECHNIQUE

<table>
<thead>
<tr>
<th>Group</th>
<th>No tested</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natives of Altamira</td>
<td>123</td>
<td>4.8</td>
</tr>
<tr>
<td>Residents at agropolis of Km 46 for about 1 month</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Natives from Belém</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Patients with R.A.</td>
<td>18</td>
<td>94</td>
</tr>
<tr>
<td>Familial contacts</td>
<td>9</td>
<td>55</td>
</tr>
</tbody>
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(W.R.A.I.R.) on paired sera from representative patients was negative for 17 arbovirus strains by the haemagglutination-inhibition test (except for one seroconversion to Catu virus after onset of purpura); 18 lepto-spiral serogroups (microagglutination technique); and spotted fever typhus, typhus group, scrub typhus, and Q-fever rickettsial groups (indirect fluorescent antibody test). In 1973, W.R.A.I.R. examined the sera from 5 patients for anti-platelet antibodies. None was found.

Sera from patients, contacts, and other sources were examined by Ouchterlony double-diffusion-in-gel technique, for precipitating antibodies to an extract of black-flies captured in the H.S.A. focus. The extract was made by suspending a macerate of approximately 300 simulidrein 1 ml of "tris" (hydroxymethyl) aminomethane buffer, followed by centrifugation for fifteen minutes at 2000 g (see accompanying table). Whereas 94% of the patients and 55% of their contacts (other immigrants living in the area) possessed precipitating antibodies against the flies, only 4-8% of the natives of the Altamira region were positive and no positive sera were found among residents of Belém, where there are no black-flies. Sera from 8 colonists who had resided in the region less than a month were also negative. Serological testing of paired sera from natives of Altamira, whereas two lines were seen in most patients. In fact, serum from one patient gave three lines. Precipitating antibodies could be detected as early as six days after onset of the disease and persisted from nineteen days up to four months.

Discussion

Despite exhaustive interviews and laboratory investigations, we were unable to relate the cases observed to a specific aetiological agent. Exposure to drugs, toxic chemicals, contaminated food, and urti-carious caterpillars was denied by all patients interviewed. Attempts to isolate a specific infectious agent from clinical and necropsy specimens by culture and inoculation of laboratory animals were uniformly negative. Serological testing of paired sera against a panel of pathogenic agents known to produce haemorrhagic phenomena in man (leptospira, rickettsia, arbo-virus) was similarly non-productive, both at the Evandro Chagas Institute and at the W.R.A.I.R.

Clinically these cases did not resemble any known infectious haemorrhagic disease. Fever was virtually absent, as were shock, jaundice, neurological manifestations, and evidence of nephritis. Epidemiology and serology suggested an association between this haemorrhagic disorder and black-fly biting.

Immigration to the Altamira region began in July, 1971, and the first cases of the haemorrhagic syndrome were reported in January, 1972. The rainy season in this area begins in December and by January the simulidre populations are greatly increased and biting voraciously, according to all colonists interviewed. Natives of the Altamira area recalled similar sporadic cases in the past, but only in immigrants. The immigrants settling in the Altamira area had come primarily from north-east Brazil, whereas black-flies do not seem to be a serious problem. All of the 92 cases recorded during the twenty months of observation occurred in immigrants.

In 1972 all cases occurred during the rainy season and no cases were observed in the dry season, when black-flies are absent from the area. New cases were next found in 1973, after the beginning of the rainy season and the reappearance of Simulium. Two peaks in the number of cases were seen, one in February, 1973, and the second in May, 1973. Each of these peaks numbers of Simulium captured from human bait were the highest recorded in the studies conducted from November, 1972, to August, 1973. Conversely, one month before the drop in cases, recorded in March, 1973, there was a pronounced fall in the black-fly-capture rate. The same phenomenon was observed before the fall in the number of cases reported in June, 1973.

Serum from patients and other immigrants formed 2–3 precipitation bands against extracts of whole simulidre. Serum from persons with little or no exposure to black-fly bites occasionally formed one band. Attempts to detect anti-platelet antibodies in patients’ sera were unsuccessful, possibly because extracts of macerated black-flies were not included in the test system.

The evidence reviewed above suggests that this haemorrhagic syndrome may be produced by a hypersensitivity phenomenon or response to a toxin associated with intense black-fly biting. Either a direct toxic effect or a hypersensitivity reaction could lead to an arrest of megakaryocyte maturation, as seen in the bone-marrow of two patients. Direct action of a toxin could produce the local cutaneous haemorrhages surrounding the sites of the black-fly bites. Other experiments are planned, including attempts to develop an animal model for study of this syndrome. An intensive surveillance system has been implemented to facilitate identification and investigation of human cases and an expanded programme of ecological field studies has begun.

We thank Dr Aaron Alexander, Dr Bennett Ellisberg, and Dr Joel Dalrymple of the W.R.A.I.R. for some of the laboratory studies; Dr Leonidas B. Dias for histopathology; Dr Halh Fraile for Simulium taxonomy; and Dr Craig Llewellyn, Dr G. H. G. Eisenberg, Jr., and Dr Ralph Lainson for assistance with the paper. This study was supported by the National Research Council of Brazil, Superintendencia de Valorizacao da Amazonia, Pan-American Health Organization, and the U.S. Army Medical Research and Development Command.

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