Memoranda
Mémorandums

Memoranda are statements concerning the conclusions or recommendations of certain WHO scientific meetings; they are signed by the participants in the meeting.

Les Mémorandums exposent les conclusions et recommandations de certaines réunions scientifiques de l'OMS; ils sont signés par les participants à ces réunions.

Bulletin of the World Health Organization, **64** (4): 511–524 (1986)

Present status of yellow fever: Memorandum from a PAHO Meeting*

An international seminar on the treatment and laboratory diagnosis of yellow fever, sponsored by the Pan American Health Organization (PAHO) and held in 1984, differed from previous meetings on yellow fever because of its emphasis on the care and management of patients and because the participants included specialists from several branches of medicine, such as hepatology, haematology, cardiology, infectious diseases, pathology and nephrology. The meeting reviewed the current status of yellow fever and problems associated with case-finding and notification; features of yellow fever in individual countries of Latin America; health services and facilities for medical care as they relate to diagnosis and management of cases; prevention strategies for and current status of immunization programmes; clinical and pathological aspects of yellow fever in humans; pathogenesis and pathophysiology of yellow fever in experimental animal models; clinical and specific laboratory diagnosis; treatment of the disease and of complications in the functioning of individual organ systems; prognosis and prognostic indicators; and directions for future clinical and experimental research on pathophysiology and treatment.

Despite the availability of a highly effective vaccine, yellow fever remains an important cause of morbidity and mortality in large parts of the tropical areas of Africa and the Americas, its true incidence being significantly underestimated in official reports. Management of patients with yellow fever has changed little in 50 years, and there are no clear therapeutic guidelines based on an understanding of pathophysiological mechanisms. The occurrence of rapid regeneration and complete healing of the liver in patients who survive suggests that appropriate measures to deal with shock, electrolyte imbalance, renal failure, and the complications of severe infection may reduce mortality. It may be noted that yellow fever has some features in common with the related flaviviral disease, dengue haemorrhagic fever/shock syndrome (DHF/DSS), in which intensive care and fluid administration are life-saving.

CURRENT STATUS

In the Americas

Yellow fever remains confined to the forested areas of the Amazon, Orinoco, Catatumbo, Atrato, and Magdalena river basins, where it affects unvaccinated persons, mainly adult males engaged in agricultural work. The virus is maintained in a cycle involving nonhuman primates and tree-hole breeding mosquito vectors of the *Haemagogus* genus. Transovarial transmission of the virus in *Haemagogus* spp. has been shown to occur in laboratory experiments and may help to explain virus survival during extended dry seasons. Little research on yellow fever ecology

* This Memorandum is based on the report of an international seminar sponsored by the Pan American Health Organization (PAHO) and held in Brasilia, Brazil, on 2–6 April 1984. The list of participants is given on pp. 523–524. Requests for reprints should be addressed to Dr Francisco P. Pinheiro, Pan American Health Organization, 525 Twenty-third St N.W., Washington D.C. 20037, USA. A French translation of this Memorandum will appear in a later issue of the *Bulletin*.

Table 1. Number of yellow fever cases reported in Africa and the Americas from 1965 to 1983

	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983
Africa:																			
Angola							65												
Burkina Faso					87														356
Cameroon						1		2	1	1	2	1				7			
Congo					1	2													
Côte d'Ivoire																		25	
Equatorial Guinea						4													
Ethiopia		350																	
Gambia														30					
Ghana					5	12	3	5	5	1	2	2	110	219	494	8	4	6	372
Liberia			5																
Mali					21														
Nigeria					208	4			2	25					11	1			
Senegal	243														3		3		
Sierra Leone											130								
Zaire							2												
Total	243	350	5	0	322	23	70	7	8	27	134	3	110	249	508	16	7	31	728
Americas:																			
Argentina	2	51	1																
Bolivia	19	69		27	8	2	8	9	86	12	151	19	2	11	10	46	102	95	11
Brazil	14	167	2	2	4	2	11	12	70	13	1	1	9	27	12	26	22	24	6
Colombia	2	3	5	11	7	7	9	3	16	36	12	22	9	104	51	7	7	1	1
Ecuador			1								3	1		1	14	2	2		5
Guyana				1															
Panama										4									
Paraguay										9									
Peru	45	9	3	5	28	75		7	33	2	1	1	82	82	97	25	98	17	27
Suriname				1	1			2											
Trinidad and Tobago															18				
Venezuela	5	5						22	7					3	3	4			
Total	87	304	12	47	48	86	28	55	212	76	168	44	102	228	205	110	231	137	50

has been done in the Americas in the past 25 years, however, and important questions remain regarding vectors and vertebrate hosts.

During the past 19 years (1965–83), 2230 cases of sylvatic yellow fever were reported to the Pan American Health Organization (Table 1). The annual incidence ranged from 12 cases (in 1967) to 304 cases (in 1966), with peaks of epizootic activity every 8 to 10 years. These epizootic waves are asynchronous from country to country. Official reports under-

CORRIGENDUM

Vol. 64, No. 4 (1986)

Present status of yellow fever: Memorandum from a PAHO Meeting

Page 512, Table 1:

We have just been informed about errors in some of the figures in Table 1. The corrected table is given below; the bold figures are the corrected ones.

Table 1. Number of yellow fever cases reported in Africa and the Americas from 1965 to 1983

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Africa:																			
Angola							65												
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Equatorial Guinea						4													
Ethiopia		350																	
Gambia														30					
Ghana					5	12	3	5	5	1	2	2	110	219	494	**9**	4	6	372
Liberia			5																
Mali					21														
Nigeria					208	4			2	25					11	1			
Senegal	243														**1**		3		
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Total	243	350	5	0	322	23	70	7	8	27	134	3	110	249	**506**	**17**	7	31	728
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Brazil	14	167	2	2	4	2	11	12	70	13	1	1	9	27	12	**27**	22	24	6
Colombia	2	3	5	11	7	7	9	3	16	36	12	22	9	**105**	51	**11**	7	**2**	1
Ecuador			1								3	1		1	14	2	2		5
Guyana				1															
Panama										4									
Paraguay										9									
Peru	45	9	3	5	28	75		7	33	2	1	1	82	**93**	97	**30**	98	**19**	27
Suriname				1	1			2											
Trinidad and Tobago															18				
Venezuela	5	5						22	7					3	3	4			
Total	87	304	12	47	48	86	28	55	212	76	168	44	102	**240**	205	**120**	231	**140**	50

estimate the magnitude of the problem 10- to 20-fold, since they largely take account of only the fatal cases detected by viscerotomy.[a] Underreporting also reflects a low index of suspicion for the disease, the limited access to medical facilities in remote areas, and the difficulty in obtaining diagnostic laboratory tests.

Although urban (*Aedes aegypti*-borne) yellow fever has not occurred in continental South America since 1942, the risk because of urbanization was considered to have increased in recent years. Human populations in the enzootic area have increased and *Aedes aegypti* has reinvaded several countries from which it had previously been eradicated, and also invaded high altitudes and rural locations of Colombia, where it was never found before. The situation is complicated owing to the development of insecticide resistance, relaxation of controls and eradication measures in some areas, and the increasing cost of these measures.

In Africa

Yellow fever is endemic or epidemic in 29 countries of the tropical zone between 15°N and 10°S. The virus-vector relationships are complex and differ with geographical location. In the rain forest areas, *A. africanus* is responsible for enzootic transmission between nonhuman primates, and occasionally for human infections in persons invading this habitat. The moist savanna regions of West and Central Africa have been termed the yellow fever "emergence zone" by Germain et al. (*1*), for here tree-hole breeding of *Aedes* populations and virus transmission increase during each rainy season. Antibody prevalences in adults may exceed 50% and the annual incidence of infection 1%. Areas at the fringe of the zone may escape virus activity long enough for a susceptible human population to accumulate, epidemics occurring when the virus is introduced during ecologically favourable periods. Outbreaks of this sort principally affect the children in the population. In dry savanna areas, where water storage is practised and peridomestic *A. aegypti* is the only abundant vector, yellow fever is not recurrently active and natural human immunity prevalences are absent or low. Introduction of the virus to such areas may be followed by explosive epidemics of the "urban" type. Large towns and cities in Africa infested with *A. aegypti* are also potentially at risk of urban yellow fever. The diagnosis, surveillance and prevention of the disease in Africa, and the management of cases and epidemics, have recently been described in the book *Prevention and control of yellow fever in Africa*, published by WHO in 1986.

In contrast to the situation in the Americas, an active ecological research effort has been continually under way for many years in three francophone countries, Senegal, Ivory Coast, and the Central African Republic, sponsored by the Institut Pasteur and the Office de Recherches Scientifiques et Techniques d'Outre Mer (ORSTOM). Among the important contributions of these groups has been the demonstration that yellow fever virus is maintained over the extended dry season by transovarial transmission in tree-hole breeding *Aedes* (*2*). Yellow fever virus has also been isolated from adults and eggs of *Amblyomma* ticks collected in the field (*3*), which raises the possibility that other vectors may play a role in virus maintenance or dissemination.

Between 1965 and 1983, 2841 cases of yellow fever were officially reported from Africa, but these represent a small fraction of the real number of cases. For example, in the Gambia in 1978, where 30 cases and 30 deaths were notified, a visit to 9 villages led to the identification of 271 clinically suspect cases of which 94 were confirmed by laboratory tests. Serological studies indicated that approximately 8400 cases and 1600 deaths occurred in the Gambia during the epidemic (*4*). In 1983, outbreaks occurred in Ghana and Burkina Faso; 315 cases (166 fatal) and 356 cases (286 fatal) were notified respectively, but the true incidence is believed to have been considerably higher. The disease affected children, mainly in the 0–4-year age group, who were unprotected by vaccination or cross-protective heterologous flaviviral antibodies.

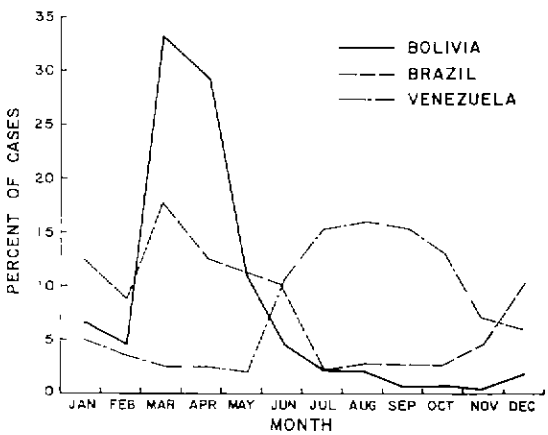

Fig. 1 Seasonal distribution of yellow fever cases in three Latin American countries.

[a] This method of surveillance, first developed in Brazil in 1930, is based on post-mortem collection of liver samples for histopathological examination, often by the use of a specially-designed trocar (viscerotome).

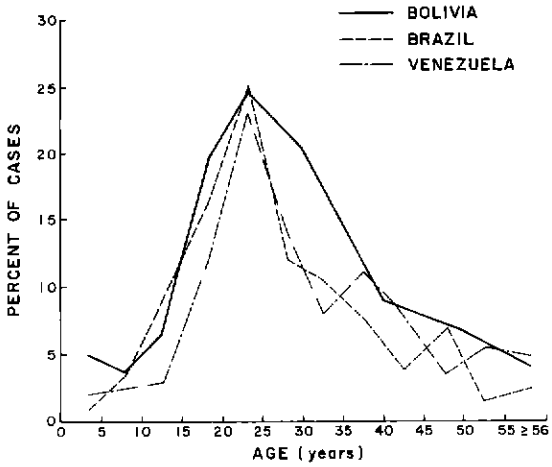

Fig. 2. Age distribution of yellow fever cases in three Latin American countries.

In individual countries of Latin America

The comparative epidemiology of sylvatic yellow fever in four countries (Bolivia, Brazil, Colombia, and Venezuela) exhibits a commonality of features. The disease occurs at highest frequency during the late rainy season (Fig. 1) when vector population densities are high and people are engaged in clearing forests in preparation for planting. The seasonal distribution of cases differs from country to country, depending on latitude and other factors affecting the onset of the rainy season.

Age, sex, and occupation are important risk factors. The majority of cases are in young adults between 15 and 40 years of age (Fig. 2), and males are affected four times more often than females. The age and sex distribution is explained by occupational exposure during agricultural pursuits (Table 2). Unvaccinated colonists who migrate from nonendemic to endemic areas constitute a high-risk group. In some areas, however, the age and sex distribution has varied. In 1981, in Rincon del Tigre, Bolivia, where women and children played a significant role in agricultural development, approximately 25% of the yellow fever cases were in children aged under 10 years, and the male:female case ratio was 1:1.3.

Surveillance of human yellow fever relies almost entirely upon a "passive" system of collection of liver samples from fatal cases for histopathological examination. Surveillance of monkey populations is conducted in some areas, a useful procedure if deaths are found, but meaningless if they are not, since yellow fever transmission can easily escape detection. In several countries, neither viscerotomy nor monkey surveillance is as widely practised today as in past years. Surveillance of *A. aegypti* remains an extremely important activity, especially in areas at risk of reintroduction of this species.

Vaccination is the sole means of prevention of sylvatic yellow fever. Both fixed and mobile vaccination units are employed. Well over 100 million vaccinations have been performed since the 1940s and vaccination coverage of populations at risk exceeds 85% in some areas. Vaccination of migrant labourers, the most vulnerable population group, remains a logistical problem. Extension of immunization coverage to large cities infested with *A. aegypti* has been necessary when yellow fever appeared in nearby forests.

Table 2. Occupations of yellow fever cases in Venezuela, 1941–1983

Occupation	No. of cases
Agricultural and cattle workers	111 (55.5)[a]
Road workers	32 (16)
Timber workers	20 (10)
Students	3 (1.5)
Truck drivers	3 (1.5)
Oil workers	2 (1)
Topographers	2 (1)
Hunters	2 (1)
Balata[b] workers	2 (1)
Businessmen	1 (0.5)
Unspecified	22 (11)
Total	200 (100)

[a] Figures in parentheses are percentages.

[b] A tree whose latex-like sap is used as a substitute for gutta-percha.

Health services

Cases usually occur in remote villages at the periphery of the health service infrastructure and are excluded from access to intensive care facilities. Patients are seen first by medical personnel at rural outposts or dispensaries with limited diagnostic and therapeutic capabilities. The problem is compounded by the difficulty of recognizing the diagnosis during the early phase of infection and in instances of sporadic or isolated disease in areas where viral hepatitis, malaria, and leptospirosis are endemic. Evaluation of the severity of a case of suspected yellow fever, an extremely important task in making a

decision to evacuate the patient to a referral facility, is also difficult because early markers of poor prognosis have not been defined and because it is difficult to obtain laboratory testing. District hospitals serving remote areas where outbreaks occur are generally poorly equipped for treatment requiring intensive care. Evacuation of patients to more important medical centres may engender not only more risk to the severely-ill patient, but also a risk to the community if *A. aegypti* is present.

Prevention strategies

In the Americas, yellow fever 17D vaccine has been systematically administered at fixed centres and by mobile units since the late 1930s. Although some Indian populations in remote areas remain unimmunized, the most vulnerable group comprises agricultural labourers engaged in colonization enterprises, who migrate from nonendemic to endemic areas. The reinfestation by *A. aegypti* of areas of Colombia, Brazil, and Bolivia close to forests where yellow fever virus is enzootic creates new population groups at potential risk of infection and epidemic spread.

Priorities for vaccination have been based on surveillance and assessments of risk on the basis of ecological and epidemiological factors. Community action and public relations are important components of the success of vaccination programmes. Immunization should be continuous in nature, with revaccination of the stable population at 10-year intervals; where there is a high risk of infection or migration of large numbers of non-immune persons, more frequent vaccination cycles are necessary. Programmes ideally aim at 100% coverage. Evaluation of vaccination coverage, monitoring of the cold chain, and confirmation of the potency of vaccines used in the field are important components of yellow fever immunization programmes.

In Africa, the situation is considerably more hazardous than it was during the 1940s and 1950s when mass immunization was routinely practised in the francophone countries of West and Equatorial Africa. As yellow fever gradually disappeared, interest in the disease declined and vaccination programmes ceased. Despite the re-emergence of severe epidemics in the 1960s, mass campaigns have generally been conducted only following major outbreaks, often initiated after the epidemics have ceased. The WHO Expanded Programme on Immunization (EPI) provides a means to resume systematic vaccination in Africa, and four countries have included yellow fever vaccination in their programmes.

Problems relating to yellow fever vaccine itself were identified. These include: (1) the thermolability of the vaccine and the requirement for a cold-chain for transport and storage; (2) the limited production capability and potential shortage of vaccine in the event of large-scale outbreaks or wider use in EPI; and (3) the increasingly antiquated vaccine production facilities and methods of preparation in embryonated chicken eggs. Improved thermostability of the vaccine has been achieved by several producers and research has begun on alternative approaches to production of yellow fever vaccines in cell culture substrates as well as by genetic engineering. In addition, modernization of the vaccine production facility in Brazil has recently been accomplished.

HUMAN YELLOW FEVER: CLINICAL AND PATHOLOGICAL ASPECTS

Yellow fever shares clinical features with other viral haemorrhagic fevers (HF) (e.g., dengue HF, Crimean HF, and Lassa fever), but is characterized by more severe hepatic involvement. Classical descriptions of severe yellow fever emphasize three clinical periods: the periods of infection, remission,

Table 3. Clinical periods of yellow fever and their principal manifestations

Day	Clinical period	Symptoms	Signs	Laboratory findings
0–3	Infection	Sudden onset, fever, headache, lumbosacral pain, nausea, vomiting, lethargy	Fever, conjunctival injection, abdominal tenderness, relative bradycardia	Viraemia, leukopenia
3–4	Remission	Reduced fever and symptoms, lasting several hours or more		
4–10	Intoxication	Fever, vomiting, bleeding, weakness, anxiety	Jaundice, oliguria, haemorrhage, hypotension, shock, agitation, prostration, stupor, coma	Viraemia wanes, antibodies appear, abnormal liver function tests, albuminuria, azotaemia, abnormal coagulation tests, hypoglycaemia, ECG changes

Table 4. Major histopathological features of human yellow fever

1. Liver	Coagulative necrosis of hepatocytes Sparing of cells around central veins and portal areas (midzonal necrosis) Councilman bodies Microvesicular cytoplasmic fat accumulation Eosinophilic intranuclear inclusions (Torres bodies) Pigment deposition in Kupffer cells (Villela bodies) Minimal or absent inflammation Reticulin framework largely preserved; no post-necrotic fibrosis
2. Kidney	Cloudy swelling, fatty change, and necrosis of tubular epithelium Granular and hyaline intraluminal casts Bile staining of tubular epithelium Schiff-positive transformation of glomerular basement membrane No inflammation; no post-necrotic fibrosis
3. Heart	Cloudy swelling, degeneration, fatty infiltration, necrosis of myofibres, including sinoauricular node and auriculoventricular bundle
4. Brain	Perivascular petechial haemorrhages, erythrodiapedesis Perivascular and interstitial oedema

and intoxication (Table 3). The case-fatality rate (CFR) of severe yellow fever is approximately 50%; if all cases with jaundice comprise the denominator, the CFR is 20%; and if all infections, including mild forms, are included, it is about 5%.

Hepatic involvement. The liver is the principal target organ in yellow fever. Microscopic examination reveals coagulative necrosis of hepatocytes and other changes listed in Table 4. The proportion of the lobule affected varies from 5% to 100%, with a mean of 80%.

In fatal cases, elevated serum bilirubin levels appear as early as the third day, increase rapidly, and peak on the sixth to eighth day when the patient's condition is critical. In nonfatal cases, jaundice appears later and declines rapidly. In some surviving cases, however, prolonged hyperbilirubinaemia may persist for a month or more during convalescence. It is not known whether such patients have underlying chronic liver disease, exacerbated by acute yellow fever virus infection.

Other liver function test abnormalities indicate hepatocellular injury but do not present a specific or diagnostic pattern. Aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) levels rise on the second or third day, peak on days five to eight, and in surviving patients decline rapidly by day 16; slight elevations may remain for up to two months. Fatal cases have mean levels two to three times higher than nonfatal icteric cases (*5*). Other enzymes reflecting hepatocellular injury are also elevated, whereas alkaline phosphatase levels are normal or minimally raised.

Extrahepatic manifestations of liver injury are widespread and affect multiple organ systems (Table 5). Injury to reticuloendothelial and biosynthetic elements of the liver underlie, at least in part, the haemostatic, cardiovascular, metabolic, renal, and neurological derangements in the disease.

Coagulopathy. The bleeding diathesis in yellow fever may be quite severe, coffee-grounds haematemesis being a typical feature. Laboratory abnormalities found in human patients include prolonged prothrombin, partial thromboplastin, and clotting times. Reductions in levels of clotting factors (II, V, VII, IX, and X) synthesized by the liver may be less than 25% of normal. In some patients, diminished concentrations of factor VIII and fibrinogen, thrombocytopaenia (*6, 7*), and presence of fibrin split products favour a diagnosis of disseminated intravascular coagulation.

Renal dysfunction. This is manifested by albuminuria and oliguria in milder forms of the disease and by virtual anuria and azotaemia in severe yellow fever. Fatal cases have mean blood urea concentrations of 109 mg/dl and creatinine levels of 5.9 mg/dl; surviving cases have lower mean levels (59 and 2.6 mg/dl, respectively). Some patients who survive the acute hepatic phase of yellow fever later die of

Table 5. Extrahepatic manifestations of liver injury in yellow fever

Haemorrhage	Decreased synthesis of vitamin K-dependent clotting factors Disseminated intravascular coagulation
Hypoglycaemia	Decreased glycogenolysis and gluconeogenesis ?Decreased insulin clearance
Hypotension	?Impaired detoxification of vasoactive substances, endotoxin ?Decreased hepatic blood flow, portal sequestration Haemorrhage
Metabolic acidosis	Hypotension Impaired carbohydrate metabolism Acute renal failure
Renal failure	Hypotension, decreased effective renal blood flow
Encephalopathy	Metabolic derangement ?Impaired detoxification of neuroactive substances ?Decreased cerebral blood flow

acute tubular necrosis or its complications, including bacterial infections.

Cardiovascular involvement and shock. Sinus bradycardia, inconsistent with the presence of fever (Faget's sign) and electrocardiographic changes, including prolongation of the PR and QT intervals, are features of acute yellow fever. Hypotension and shock appear as late events in the course of severe infection. Although many physiological mechanisms may be involved in the genesis of shock, myocardial injury may play a significant contributory role. The occurrence of sinus bradycardia, possibly reflecting lesions in the sinoauricular node and bundle of His, would appear to be deleterious in the face of hypotension. Late deaths, occurring in the convalescent phase, have been attributed to cardiac failure or arrythmia but remain poorly documented.

Encephalopathy. The terminal stage of human yellow fever is characterized by agitation, mania, delirium, convulsions, and coma. Although a single case of brain infection by yellow fever virus has been described (*8*), the neurological abnormality is usually the result of metabolic encephalopathy and cerebral oedema without evidence of inflammation or direct viral injury. Cerebral oedema is a frequent cause of death in patients with acute hepatic failure, and has been documented in patients dying of yellow fever. Other features of yellow fever which may contribute to encephalopathy include shock, metabolic acidosis, respiratory depression and hypercapnia, renal failure, and hypoglycaemia.

EXPERIMENTAL YELLOW FEVER

Animal models. In laboratory mice yellow fever is a neurotropic infection. The only small animal which manifests a viscerotropic response to infection, with histopathological features similar to those in humans, is the European hedgehog (*Erinaceus europaeus*), but this interesting model has not been studied for over 40 years (*9*).

The pathogenesis of yellow fever in rhesus and cynomolgus monkeys is similar to that in humans, but the course of infection is more rapid. Over 600 monkeys, mainly rhesus, have been used in published studies of yellow fever over the years. Each of these studies has focused on a single or a few parameters rather than on a comprehensive investigation of host responses. Future research should involve a multidisciplinary approach to the definition of multiple organ system dysfunction at the biochemical and physiological levels.

Viral variation. Considerable heterogeneity has been reported with respect to T_1-resistant RNA oligonucleotide fingerprints, neurotropism for mice, virulence for monkeys, and antigenic markers of wild strains of yellow fever virus. However, there have been no systematic studies of variation in viscerotropic virulence, ability to infect genetically defined strains of vector mosquitos, or geographic virological markers. The viral genes responsible for determining tissue tropism and virulence have not been defined. Recent advances in gene sequence analysis of 17D virus should allow comparative molecular studies with wild viruses.

Sites of viral replication other than the liver have not been defined in human cases, nor have definitive studies been conducted in monkeys. Serial sacrifice studies have been flawed by use of an unnatural (intraperitoneal) route of inoculation and the difficulty in separating the contribution of virus in blood to tissue infectivity. Early work indicated that the virus replicates in lymph nodes draining the site of inoculation, followed by haematogenous spread to and growth in many tissues, including liver, spleen, striated muscle, kidney, and bone marrow (*11*). In one sequential histopathological and immunofluorescence study, early lesions and antigen were detected in Kupffer cells in the liver and in scattered reticuloendothelial cells in the spleen and lymph nodes (*11*). Yellow fever virus has been shown to replicate in monocytes, phytohaemagglutinin-stimulated lymphocytes, and macrophage-like cell lines (*12*) as well as in macrophages and lymph nodes *in vivo*. In future studies, antigen-detection methods or nucleic-acid probes should be used to systematically determine the chronological sequence of viral replication in tissues and to define the cell types involved.

Hepatic involvement. The liver is the principal target organ in experimental monkeys as in humans. Kupffer cell infection is established 24 hours after inoculation and acidophilic necrosis of Kupffer cells, along with antigen demonstrable by immunofluorescence, precedes hepatocellular degeneration. Hepatocellular changes are minimal until the last 24–36 hours before death, when necrosis proceeds very rapidly.

Renal failure. Biopsies of the kidneys of rhesus monkeys show no abnormalities until 24 hours before death, and tubular necrosis occurs as a terminal event. Changes in renal function occur mainly within the terminal 24–36 hours, when urine output decreases, serum creatinine rises, and there is a marked drop in urinary Na^+ excretion (*13*). At its onset, renal failure thus reflects a functional decrease in glomerular filtration rate and renal plasma flow. Shortly before death, the onset of arterial hypotension and renal ischaemia leads to tubular necrosis.

These findings indicate that early treatment of functional (prerenal) failure may prevent tubular necrosis, and they should be confirmed by experimental and clinical studies.

Coagulopathy. A study published over 15 years ago documented the occurrence of disseminated intravascular coagulation in rhesus monkeys infected with yellow fever (*14*). Heparin therapy reversed the laboratory abnormalities but did not alter the course or outcome of infection.

Myocardial and haemodynamic function. Cardiac muscle is a major site of flaviviral replication (*15*). The lesions observed in the myocardium of experimentally-infected monkeys (granular degeneration and necrosis of muscle fibres (*16*)) are undoubtedly mediated by direct viral injury. Lesions may involve the sinoauricular node, accounting for sinus bradycardia, prolonged conduction time (PR interval), AV block, and ST-T wave abnormalities. Decreased arterial blood pressure and pulse pressure as well as bradycardia have been observed during the terminal stages of experimental yellow fever (*13*). Prior to this terminal phase, blood flow to specific organs, notably the kidneys, may be reduced despite normal aortic pressure. Changes have been documented in the water and electrolyte content of cardiac muscle of infected rhesus monkeys (*17*). Tissue Ca^{++} levels were significantly decreased and extracellular K^{+} increased. Such changes could impair systolic pump function and contribute to shock in yellow fever.

Human patients with dengue haemorrhagic fever/shock syndrome (DHF/DSS) have been found to have increased peripheral vascular resistance and diminished cardiac output due to diffuse capillary leakage of plasma proteins across capillaries, hypovolaemia, and decreased venous return. Whether similar mechanisms explain hypotension in yellow fever is unknown. In one study (*17*), yellow fever infected monkeys had increased blood and plasma volumes and decreased haematocrits in distinct contrast to humans with DHF/DSS. Additional studies of cardiac function, peripheral vascular integrity, and intravascular volumes are needed to clarify the pathophysiology in the monkey model and to establish guidelines for human studies.

Acid-base and electrolyte disturbances. Tissue hypoxia, lactic acidosis, and hyperkalaemia are probable terminal events in both monkeys and human patients. In an experimental study of monkeys, a decrease in pCO_2 (respiratory compensation for metabolic acidosis) was present 12–48 hours before death, arterial pH was modestly decreased, and in the final two hours, severe combined respiratory and metabolic acidosis and hyperkalaemia were present (*13*).

Encephalopathy. In experimentally infected monkeys, depression, stupor and coma occur during the terminal phase of infection. Despite high viraemias, the virus does not invade the brain, and CNS changes appear to be metabolic in origin. Contributing factors are hypotension, acidosis, and hypoglycaemia, but signs of CNS depression often precede these abnormalities. Changes in brain tissue concentrations of water and electrolytes, noted in one study (*17*), may adversely affect function.

DIAGNOSIS

The diagnosis of patients during the period of infection or while having atypical, mild forms of yellow fever is practically impossible on clinical grounds alone, even during epidemics. A number of infectious and noninfectious diseases must be considered in the differential diagnosis of severe yellow fever with jaundice (Table 6).

The use of monoclonal antibodies and enzyme immunoassays has provided new approaches to the early, rapid diagnosis of yellow fever. These techniques should be made widely available as a means to improve surveillance, care, and management of patients, and control of spread. It is now possible to detect yellow fever viral antigen (*18*) and/or IgM antibodies (*19*, *20*) during the first week of illness by methods which may be performed under field conditions.

In laboratories equipped to maintain cell cultures, advantage can be taken of the presence of virus-IgM

Table 6. Differential diagnosis of severe yellow fever

Parasitic infections:
Falciparum malaria
Bacterial infections:
Typhoid fever
Leptospirosis
Tick-borne relapsing fever
Rickettsial infections:
Typhus
Q fever
Viral infections:
Viral hepatitis, especially fulminant hepatitis B, and delta agent
Labrea hepatitis[a]
Rift Valley fever
Congo-Crimean haemorrhagic fever
Other haemorrhagic fevers (Lassa, Marburg, Ebola, Bolivian haemorrhagic fever)
Surgical, drug-induced, toxic conditions

[a] See references *27–30*.

antibody complexes (normally noninfectious for mosquito cell cultures) in many patients' sera. Immune complexes may be rendered infectious by degrading the IgM with dithiothreitol, thereby releasing infectious virus.

Recent success in cloning and sequencing the genome of yellow fever 17D virus (*26*) should allow the development of sensitive new methods for detecting the yellow fever viral genome in clinical specimens by nucleic acid hybridization.

Histopathological examination of post-mortem liver specimens remains an important diagnostic procedure, especially in Latin America. Characteristic lesions are seen mainly in livers obtained before the eighth day of illness, and pathological specificity decreases thereafter. Detection of yellow fever antigen in formalin-fixed liver by immunoperoxidase staining has been reported (*21*) and requires confirmation. It is likely that the detection of antigen by immunoassay or of viral genome by nucleic acid hybridization will prove useful as an adjunct to histopathological examination of post-mortem material. Needle biopsy of the liver, a hazardous procedure because of the risk of haemorrhage, is contraindicated.

TREATMENT

Although very few patients with yellow fever have been treated in well-equipped medical centres, evidence from a limited number of studies, largely unpublished, suggests that intensive care can reduce complications and mortality. In developing their report, the participants of this meeting drew on experience in the treatment of other diseases that appear to share clinical or pathophysiological features with yellow fever, and they emphasized the measures that are available in relatively well-equipped hospitals. As the occasion arises in the future, it will be essential to document and publish observations on the intensive care of yellow fever patients.

Liver failure. Treatment should be initiated at the earliest sign of significant hepatic decompensation, prolongation of the prothrombin or partial thromboplastin time to twice normal being the most sensitive marker of a breakdown in the liver's synthesizing functions. Treatment of hypotension was emphasized because hypoperfusion and oxygenation may aggravate the liver injury. Maintenance of adequate nutrition and prevention of hypoglycaemia by intravenous administration of 10–20% glucose solutions was recommended, but with care to prevent fluid overload. Nasogastric suction was considered essential to prevent both hypotension due to gastric distension and aspiration of gastric contents. Drugs which act on the central nervous system, including phenothiazines, barbiturates, and diazepam derivatives should be avoided, since they may precipitate or aggravate encephalopathy.

Cardiovascular involvement and shock. Careful initial and repeated evaluations of fluid balance and cardiovascular status are required in all patients during the period of intoxication. Those who develop signs of hypotension or hypoperfusion require continuous monitoring, the best environment for such patients being an intensive care unit.

In patients with manifestations of hypotension, a principal goal is identification of decreased circulating blood volume due to excessive loss or sequestration of fluid. If hydration, oxygenation, and correction of acidosis do not correct the hypotension, or if there is evidence of a significant cardiogenic component, more sophisticated assessment and haemodynamic monitoring is necessary. Use of the Swan-Ganz catheter and direct arterial blood pressure monitoring permits fine regulation of circulating blood volume, cardiac output, and arteriovenous oxygen difference during therapy with fluid and/or vasoactive drugs. If these techniques are unavailable, frequent measurements of blood pressure and central venous pressure, as well as assessment of regional blood flow by skin temperature, cerebral status, and urine output may permit appropriate therapy. When restitution of the blood volume is not successful in raising the blood pressure to a level providing adequate organ blood flow, the administration of vasoactive drugs may be required. The agent of choice is dopamine, which combines a vasoconstrictor effect upon arterioles and veins with a renal vasodilator effect, and which also has a positive inotropic action on the heart. Dobutamine may have some advantage in yellow fever cases with bradycardia, however, because of its greater positive chronotropic effect.

Monitoring of electrolytes, arterial blood gases and pH is essential during the intensive care of yellow fever patients. Administration of oxygen is indicated not only in the presence of arterial hypoxaemia but also in low-output states with wide arteriovenous oxygen differences. Hyperkalaemia and metabolic acidosis must be corrected, if present.

Because every degree of elevation of body temperature requires a 13% increase in oxygen consumption, fever should be controlled by non-aspirin antipyretics, sponging, or a cooling blanket. Hypothermia, a frequent event in the late stages of severe yellow fever, also constitutes a threat to homeostasis, and should be treated by gentle warming.

Renal failure. In the oliguric patient, simple tests for renal tubular integrity, e.g., measurement of the

excreted fraction of filtered sodium, FE_{Na} (*22*), should be employed to distinguish between prerenal azotaemia and acute tubular necrosis. FE_{Na} is determined by simultaneous collection of plasma (p) and urine (u) for measurement of sodium (Na) and creatinine (Cr), and is calculated as follows: $FE_{Na} = [(u/p_{Na})/(u/p_{Cr})] \times 100$. In cases of prerenal failure, the FE_{Na} is less than 1%, whereas values greater than 3% are found in patients with oliguric and nonoliguric renal failure. If prerenal failure, indicating inadequate renal blood flow, is present, therapeutic emphasis is placed on monitoring and optimizing circulating blood volume and cardiac output, as discussed above. The administration of intermittent fluid challenge or the use of a potent diuretic (e.g., furosamide) may have diagnostic and therapeutic value. If a diagnosis of acute tubular necrosis is made, peritoneal or haemodialysis provides the best approach to management.

Haemorrhage. Although gastric haemorrhage is a common occurrence in yellow fever, no attention has been paid in the past to protecting the gastric mucosa. Nasogastric suction combined with use of a H_2 receptor blocker, such as cimetidine, may reduce the risk of bleeding in these patients.

Treatment of the bleeding diathesis remains controversial. Decreased synthesis of clotting factors by the diseased liver is believed to be a major factor responsible for the coagulopathy. In patients without clinical bleeding, it was considered desirable to keep the prothrombin time between 25 and 30 seconds by administration of fresh-frozen plasma, being careful not to precipitate circulatory overload. If severe bleeding occurs or the haematocrit drops, fresh whole blood should be rapidly administered in order to maintain an adequate blood volume.

Studies of a small group of patients during an outbreak in Brazil in 1972 indicated that disseminated intravascular coagulation was a major mechanism underlying the bleeding disorder (*6*, *7*). Experience during this outbreak also indicated that treatment with heparin improved the coagulation disorder and led to cessation of haemorrhage. However, the participants emphasized the complex etiology of the haemorrhagic diathesis in yellow fever and stressed the need for further studies of both experimentally-infected monkeys and human patients. Heparin should be considered only when there is convincing laboratory evidence for disseminated intravascular coagulation, and treated patients must be closely monitored clinically and by coagulation tests.

Administration of vitamin K has been advocated, but is probably ineffective in cases with fulminant hepatic necrosis; if given, it should be administered intravenously to avoid haematoma associated with intramuscular injection.

Secondary infection. Bacterial pneumonia is a frequent complication of severe yellow fever. In patients who survive the hepatitis but develop acute tubular necrosis, sepsis is a major complication and cause of death. Prompt antibiotic treatment of bacterial infections is required in these cases.

Controversial approaches. Brief consideration was given to treatment of other disease states associated with hepatic necrosis or cardiovascular shock. Glucose-potassium-insulin infusions, indometacin, lidocaine, endorphin antagonists, and glucocorticosteroids have been proposed or investigated in the treatment of shock in animal models, but their effectiveness in humans remains controversial. Corticosteroids have not proved efficacious in the treatment of DHF/DSS.

Rhesus monkeys treated with an interferon inducer (a nuclease-resistant derivative of poly-(I)-poly-(C), poly-L-lysine, and carboxymethylcellulose) 8 hours before or 8 hours after inoculation of yellow fever virus were significantly protected, but treatment delayed until 24 hours after inoculation failed (*23*). The antiviral agent ribavirin (1-β-D-ribofuranosyl-1*H*-1,2,4-thiazole-3-carboxamide), which is effective against yellow fever *in vitro*, produced no effect on viraemia or survival time in monkeys given low to moderate doses (5–30 mg/kg/day) (*24*). The usefulness of effective antiviral agents developed in the future will ultimately depend upon early rapid diagnostic techniques and good prognostic indicators.

PROGNOSIS AND PROGNOSTIC INDICATORS

The following clinical features are associated with a fatal outcome in yellow fever: (1) rapid progression to the period of intoxication and rapidly rising serum bilirubin; (2) a severe haemorrhagic diathesis and occurrence of disseminated intravascular coagulation; (3) renal failure due to acute tubular necrosis; (4) early appearance of hypotension; (5) shock; (6) coma and convulsions; and (7) intractable hiccoughs.

During the first week of illness, patients who subsequently died had serum transaminase levels two or three times higher than survivors (*5*), although there was some overlap between the two groups. The relationship between viraemia level and outcome of yellow fever infection is unknown and deserves study in the future. Both SGOT (aspartate aminotransferase) and viraemia levels have proved to be prognostically useful in Lassa fever and provide a basis for chemotherapeutic intervention. Use of rapid *in-vitro* methods for detection of yellow fever viral antigen in serum should be investigated in this context.

PRIORITIES FOR CLINICAL RESEARCH

There was general agreement on the need for in-depth studies of a series of yellow fever patients in the setting of an intensive care unit. Elucidation of the pathophysiology of yellow fever by study of a limited number of cases may identify one or more efficacious and practical therapeutic approaches which could be translated to all levels of hospital care.

Several approaches were suggested for achieving this research objective. First, improved early recognition and diagnosis of yellow fever outbreaks must be achieved by training health workers, improving surveillance and reporting, and establishing local capabilities for antigen-capture and IgM antibody-capture enzyme immunoassays.

Second, *mobile units* should be created, consisting of a laboratory technician, nurse, and physician. These units could be rapidly dispatched to an area of suspected yellow fever activity to define the basic epidemiological parameters, assist in the local care of patients, and select patients for evacuation to a referral centre or "fixed unit".

Third, *fixed units* with intensive care facilities should be established at preselected hospitals, where patients with severe infections could be optimally managed and pathophysiological research conducted. Alternatively, where large epidemics occur in remote areas, e.g., in parts of Africa, it may be necessary to bring medical expertise, monitoring equipment and clinical laboratory testing to a local or regional hospital in the affected area.

A number of specific areas for clinical research were identified:

(1) *Clinical studies on hepatic function* should include the relationships between viraemia and biochemical markers of liver injury and between liver injury, hypotension, shock, and disseminated intravascular coagulation. Another study might define insulin, glucagon and other hormones in blood during recovery since it is possible that changes in the ratio of such hormones could signal the onset of liver regeneration.

(2) *Studies on renal dysfunction* should elucidate the pathogenesis of renal failure, especially the transition from prerenal failure to acute tubular necrosis. Tests of renal tubular function (including fractional excretion of sodium, water, and amino acids; acidifying capacity; measurement of osmolarity, urinary tubular proteins, and renal blood flow) will add important data on renal injury. Search should be made for immune complexes, both in blood and in glomerular tissues. Acid-base and electrolyte balance should be continuously monitored and the efficacy of corrective therapy documented. The use of dialysis should be clarified as a means of managing the renal complications of yellow fever.

(3) *Cardiovascular studies.* Clinical, electrocardiographic, and echocardiographic observations should begin during the early phase of infection to clarify cardiac involvement in yellow fever. If bradycardia is associated with low cardiac output and hypotension in the presence of adequate blood volume, the effect of increasing heart rate with vasoactive drugs or pacing should be studied as a potential therapeutic approach. Haemodynamic studies, including measurement of cardiac output, stroke volume, and ventricular filling pressures, as well as peripheral vascular resistance and blood and plasma volumes should help to clarify the physiological alterations associated with shock in yellow fever. Increased vascular permeability and extravascular leakage of plasma proteins, central to the pathophysiology of DHF/DSS but not studied in yellow fever, require investigation. Studies should be made of the complement system and of endogenous vasoactive mediators, such as leukotrienes, prostaglandins, kinins, and histamine.

Late deaths attributed to myocardial failure or arrythmia during an epidemic in the Sudan in 1940 (*25*) have not been subsequently observed and should be investigated by appropriate follow-up of patients using non-invasive techniques (Holter monitoring, electro- and echocardiography).

Other important areas for clinical research include the coagulation disorder, the use of nasogastric suction and of cimetidine to reduce the risk of haematemesis, the chronology of humoral and cell-mediated immune responses, the role of heterologous flaviviral antibodies in cross-protection and immune enhancement, and the sites of viral replication in tissue obtained from fatal cases.

Research protocols should be developed in advance, be approved by local human studies ethical committees, and be consistent with WHO guidelines.

PRIORITIES FOR EXPERIMENTAL RESEARCH

There is a need for basic research on the replication strategy, biochemistry, and immunology of yellow fever virus. Definition of the molecular basis of virulence, cell and tissue tropism, and antigen structure and presentation will ultimately provide directions for development of new preventive or therapeutic methods.

Studies on the pathogenesis and pathophysiology of yellow fever rely on the availability of suitable animal models. Because they exhibit extreme susceptibility and a markedly accelerated course of infection, rhesus monkeys are not an ideal model of

yellow fever. Alternative primate hosts should be sought for this purpose, including neotropical species of monkeys and marmosets, and insectivores (hedgehogs) should be reinvestigated. Future studies should take a multisystem approach both to assure accumulation of comparative data and to gain maximum information from each experiment involving a nonhuman primate. The sites of yellow fever virus replication and injury should be investigated using sensitive techniques for detecting viral antigen or genetic material. The role of humoral and cell-mediated immunological responses should be elucidated both in recovery from primary infection and yellow fever challenge after heterologous flaviviral infection.

The possible contribution of bacterial endotoxin to shock and liver injury should be investigated by direct assay, use of radiolabelled endotoxin to determine leakage across the gut epithelium, use of antibiotics, and colectomy. Pathophysiological studies should be designed to elucidate the mechanisms which underlie the renal, haematological, cardiovascular, and neurological disturbances.

Specific approaches to therapy will depend upon the investigation and development of antiviral compounds, immunomodulators, or interferon. A drug screening programme at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, has identified a number of compounds active *in vitro* against yellow fever and sufficiently nontoxic to be used *in vivo* (Table 7). One of these compounds (tiazofurin or 2-β-D-ribofuranosyl-1*H*-1,2,4-thiazole-4-carboxamide) was shown to be protective in a preliminary study. Combinations of two or more agents may offer advantages over single-drug therapy. Synergistic antiviral effects against yellow fever *in vitro* has been demonstrated for the combination of ribavirin and tiazofurin. *In vitro* synergistic activity has also been shown for human interferons (alpha and gamma) against yellow fever virus. Compounds such as glucan, muramyl tripeptide, lipoidal diamine and pyram, which activate macrophages, increase host resistance against a number of viral infections. The specific delivery of muramyl tripeptide to Kupffer cells by liposomes protects mice against Rift Valley fever virus even when given 5 days after infection. Another approach that may warrant investigation is the administration of monoclonal antibodies, or the use of monoclonal antibodies incorporated in liposomal membranes as a means of targeting antiviral drugs.

Table 7. Compounds with *in vitro* efficacy against yellow fever

	ID_{50}[a]	MTC[b]	TI[c]
2-β-D-ribofuranosyl-1*H*-1,2,4-thiazole-4-carboxamide	9.2	5205	566
2-β-D-ribofuranosyl-selenazole-4-carboxamide	0.15	>1000	>6512
3-bromo-4-chloropyrazolo-(3,4-d)-pyrimidine	0.1	23.3	233
(3-β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide	3.3	>500	>151
4-amino-1-(5-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine	3.4	>500	>145
7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrrolo-(2,3-d)-pyrimidin-4-one	10.0	>500	>50
9-(β-D-ribofuranosyl)-purine-6-thiocarboxamide	23.5	>500	>21

[a] Inhibitory dose 50% (μg/ml).

[b] Minimum toxic concentration in LLC-MK2 cells (μg/ml).

[c] Therapeutic index = MTC/ID_{50}.

Recent studies have demonstrated the important role of polypeptide hormones in the regulation of hepatic regeneration, including insulin, glucagon, epidermal growth factor, platelet-derived growth factor, and some neuropeptides. These hormones act in synergy, enhance hepatocyte DNA synthesis *in vitro* and stimulate liver cell division *in vivo*. The potential role of optimal concentrations of hepatotrophic hormones in liver cell regeneration following yellow fever virus-induced injury should be investigated in animal models.

CONCLUSIONS AND RECOMMENDATIONS

Obstacles to reducing yellow fever morbidity and mortality can be categorized in two groups, those due to logistical and practical problems in applying existing knowledge and techniques, and those due to an incomplete understanding of the disease mechanisms.

Obstacles in the first group include inadequate surveillance, training of medical and paramedical personnel, notification of cases, availability of rapid specific diagnostic test procedures and accessibility of medical services. Improvements in the current status of these problem areas were recommended. Improved surveillance and reporting will depend upon a heightened level of emphasis on the part of national health authorities, specific training of personnel at all levels of health structures, and support for incorporation of specific diagnostic techniques,

particularly enzyme immunoassays for antigen and IgM antibodies. The Pan American and World Health Organizations can play a pivotal role by assisting in the transfer of this technology through workshops and in the distribution of written materials and reagents.

It was generally accepted that many patients with severe yellow fever would benefit from modern medical care aimed at reducing the morbidity associated with renal, haematological, and cardiovascular complications. Access of patients to medical services in the Americas could be significantly improved by the creation of mobile units with facilities for rapid diagnosis, early treatment and epidemiological investigation, and evacuation of cases to clinical centres with intensive care capability. The strategies for care and management of patients during epidemics in Africa is more complex. A meeting at an international level was recommended in order to define the equipment and supplies needed for a mobile therapeutic and research unit, to identify experts in clinical and laboratory medicine willing to participate, and to develop strategies for logistical implementation.

A major obstacle is the incomplete current level of understanding of the pathophysiology and pathogenesis of yellow fever. The use of mobile and fixed units, as proposed, with capabilities for conducting clinical research would provide a means for gathering fundamental information on disease mechanisms and possible therapeutic approaches. Specific clinical protocols need to be developed for this purpose and monitoring equipment and clinical laboratory test procedures must be made available.

Experiments involving animals provide an important approach to understanding the human disease. In lieu of rhesus monkeys, other animal models including new world primates should be sought. Research questions relating to sites of viral replication, mechanisms of cell injury, and physiological alterations in yellow fever have been described above. Research on the development of specific chemotherapeutic agents will be facilitated by an increased understanding of pathogenesis and improved practical animal models. Since yellow fever (as well as a number of other arthropod- and rodent-borne endemic/epidemic infections of public health importance) is not commercially exploitable, the burden of research on pathogenesis and antiviral drugs will remain with the governments and international health organizations.

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