AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 100, No. 3

Printed in U.S.A.

EVIDENCE FOR PERSISTENCE OF INFECTIOUS AGENTS IN ISOLATED HUMAN POPULATIONS[1]

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Black, F. L. (Dept. of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, Conn. 06510), W. J. Hierholzer, F. deP. Pinheiro, A. S. Evans, J. P. Woodall, E. M. Opton, J. E. Emmons, B. S. West, G. Edsall, W. G. Downs and G. D. Wallace. Evidence for persistence of infectious agents in isolated human populations. *Am J Epidemiol* 100:230–250, 1974.—More than 900 members of three Carib and four Kayapo Indian tribes, living on the periphery of the Amazon basin, have been studied for immunity to various viral, bacterial and protozoal agents. These tribes are isolated from the main Brazilian culture, and several had remained hostile and dependent on stone tools until less than 10 years prior to the study. The prevalence of antibodies to herpesvirus types 1 and 2, Epstein-Barr virus, cytomegalovirus, varicella and hepatitis B antigen was very high in every tribe studied. The age of acquisition of immunity was lower than in previously studied cosmopolitan communities. These agents seem to maintain a very stable relation with their host populations. Antibodies to measles, mumps, rubella, influenza A_0, A_2 and B, parainfluenza 1, 2 and 3 and poliovirus 1 were nearly or totally absent from one or more tribes. When these antibodies were found in anyone who had not been outside the tribial area, they were usually found in nearly everyone over a specific age. These agents seem to maintain an unstable relation with their hosts, appearing only when introduced from the outside and then disappearing again. There was no evidence of smallpox in any tribe. Antibodies to the arboviruses (yellow fever viruses, Ilhéus, and Mayaro) were found with high frequency in certain areas. Prevalence of antibody to these viruses increased gradually with age, suggesting endemicity of a different order from that of the herpes group viruses. Antibody to Toxoplasma was absent from children but was frequently present in older adults. Antibody to treponema had a very high prevalence in the Kayapo tribes without evidence of pathology, suggesting that the parasite present in these communities was well adapted to its host. Malaria and tuberculosis, on the other hand, caused extensive and severe morbidity and

Received for publication February 1, 1974, and in final form May 10, 1974.

Abbreviations: EBV, Epstein-Barr virus; FTA-ABS, fluorescent treponemal antibody absorption; HI, hemagglutination inhibition; VDRL, Venereal Disease Research Laboratory.

[1] This work was supported by a travel grant from the Pan American Health Organization and research grants from the United States Public Health Service (AI-00701 and CA-12952), The Rockefeller Foundation, and U.S. Army DADA 17-70-C-0042.

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threatened destruction of their host populations. Tetanus antibodies were virtually absent.

Indians, South American; malaria; tetanus; treponema; tuberculosis; virus diseases

INTRODUCTION

Since the studies of Panum on measles in the Faroes (1) and of Paul and Freese on the common cold in Spitzbergen (2), it has been clear that many infectious agents do not persist in small isolated communities but must be reintroduced from larger populations at the onset of every epidemic. On the other hand, Hope Simpson (3) found that varicella-zoster virus remained endemic in a small population in the Shetland Islands. Expressed in quantitative terms, it has been estimated that at least 200,000 people would be needed to sustain measles virus (4, 5), but that a population of 2000 sustained varicella virus.

Population groups of the size needed to sustain measles virus first arose with the development of urbanized societies only 4000–5000 years ago. It seems improbable that agents dependent on large aggregations of the people could have existed in their present form prior to that time. Only those microorganisms which, like varicella, can exist in small groups, and those which, like yellow fever virus, have a non-human reservoir, are likely to have played a role in the development of mankind through his much longer evolutionary history.

The purpose of this study is to determine which of our modern disease agents are able to persist in small, isolated communities. The emphasis has been placed on viruses because specific, durable serologic reactions more commonly follow virus infection than infection with more complex organisms, and these have provided our best index of past history. For this study, it was necessary to identify population groups sufficiently isolated that viruses were rarely introduced from the outside. Island populations have served this purpose in the past, but with improved means of communication, most common viruses are introduced to even the most isolated islands every few years (6–9). Mufson and associates (10–12) and Brown and Taylor-Robinson (13) have studied various isolated African tribes and found serologic evidence of recent infection with all but a few of the viruses for which they tested. In contrast, however, Neel et al. (14) found little or no influenza antibody in one South American Indian tribe and we found no serologic evidence of influenza, measles, or mumps in another such tribe (15). The present report is an extension of our earlier study (66) of seven tribes living on the periphery of the lower Amazon basin. Generally, these groups are sufficiently isolated to have been protected from many diseases. They offer a further advantage in the reconstruction of man's heritage of disease, in that they are still hunters and gatherers, as were all men through most of their evolutionary history.

When none or very few positive serologic reactions were found with a virus known to induce a long-lived response, one could not tell whether that virus was incapable of maintaining itself in the community, or whether the virus had never been introduced to the community. However, when a high proportion of the population over a specific age had antibody, while very few persons below that age gave specific reactions, it was quite certain that the agent had been introduced but that it had died out or become inactive since the birth of the youngest immune person. Conversely, if high antibody prevalence rates were found even in very young children, or if the proportion of the immune population in-

TABLE 1

Populations studied and dates of specimens

Population	No. of members	Initiation of peaceful contact	Date of specimen	Linguistic group	Reference
Ewarhoyana	14	1970	1970	Carib	16
Gorotire	400	1937	1965	Kayapo (Ge)	17
Kaxuyana	40	1725	1970	Carib	16
Kuben Kran Kegn	300	1955	1970	Kayapo (Ge)	17
Mekranoti	190	1958	1969; 1972	Kayapo (Ge)	18
Tiriyo	1000	1951	1966; 1970	Carib	19
Xikrin	123	1962	1970; 1972	Kayapo (Ge)	20
New Haven, Conn.	250,000		1957		21

creased steadily with increasing age, one could be quite confident that the virus was suriviving within the community.

POPULATIONS STUDIED AND SPECIMENS USED

The tribes under consideration varied greatly in size and in the duration of their contact with civilization (table 1). The Ewarhoyana and Kaxuyana were both remnants of much larger groups. These tribes moved to the Tiriyo village in 1970 and 1966, respectively, and it was there that the specimens were collected (figure 1).

The Xikrin tribe split from the Gorotire about 1900 and was scattered into several small groups between 1930 and 1962. The Kuben Kran Kegn represent the product of a later split of the Gorotire, having separated in 1936.

The Kaxuyana had had contact with nut collectors for many years. They had numbered about 400 persons prior to a severe measles epidemic in 1924. This epidemic left only 80 to 90 persons alive, and the tribe has never recovered its numbers. The Xikrin, too, suffered from a series of epidemics in 1962 and 1963 that reduced their population from 164 to 86. Measles epidemics were recorded in Gorotire and Kuben Kran Kegn in 1962. A rubella epidemic occurred in Tiriyo in 1963. Epidemics characterized as "grippe" had affected all the tribes except, perhaps, the Ewarhoyana, for whom our history covered a period of only a few weeks.

The home territory of the Ewarhoyana, Kuben Kran Kegn and Tiryo was savannah country with riverine forest belts. The other tribes lived in continuous tropical forest. All used a slash and burn agriculture, with manioc the dominant crop of the northern tribes and sweet potatoes most important south of the Amazon. The Gorotire, Kaxuyana and Kuben Kran Kegn villages were accessible by river most of the year, and the Xikrin were accessible for a few months. Air strips capable of handling light planes existed at each village except the Ewarhoyana and Kaxuyana. Several Indian Service and missionary personnel lived at Gorotire, Kuben Kran Kegn and Tiriyo. Single missionaries lived at Mekranoti and Xikrin at the time the collections were made.

Specimens available from the Mekranoti and the Xikrin represent essentially the whole population over one year of age of these tribes. The Tiriyo collection includes nearly all members of the tribe who lived in Brazil, plus a number of young men who visited from Surinam. The collections from the other tribes represent the members who happened to be near the central village on a particular day and these collections often under-represented young hunters. Very young children may also be under-represented in these collections.

Except for very young children, no specific data on age were available in any of these tribes. Age estimates in table 2 are based on the opinion of two or more observ-

ers and take into account the estimated ages of the relatives of each individual.

Sera from Connecticut cities are included in certain key tests as representative of modern urban communities. The New Haven collection was made before poliomyelitis, measles, mumps or rubella vaccine were distributed. The sample is balanced by social class and by age (21).

METHODS

Serologic methods were as used previously (15) except as noted below. All tests were quantitative, although only a single level was chosen as the dividing line between positive and negative in table 3.

Herpes simplex. A plaque reduction test in Vero cells was used with Nahmias' type 1 strain E 377 (22) and Gudnadottir's type 2 strain X 263 (23). Virus suspension containing 100–200 plaque-forming units per 0.1 ml was incubated with equal volumes of serial serum dilutions for 1 hour at room temperature. Two-tenths ml of the mixture was placed on drained culture sheets and incubated for 1 hour at 37 C. Cultures were fed with 5 ml Eagle's medium, with 10 per cent calf serum and with 0.2 or 1 per cent human gamma globulin (Merck "Gammagee") for types 1 and 2, respectively, and were incubated 3 days. They were then drained and were stained with 1 per cent

FIGURE 1. Map of a section of northern Brazil indicating home territory of tribes studied.

TABLE 2

Serum specimens by age (years)

Population	Total	0–4	5–9	10–19	20–29	30–39	40–49	50–59	60+
Ewarhoyana	9	0	1	2	4	2	0	0	0
Gorotire	219	7	31	54	55	41	31	0	0
Kaxuyana	27	0	5	9	5	6	1	0	1
Kuben Kran Kegn	57	10	18	8	6	6	4	1	4
Mekranoti:									
1969	95	7	9	31	18	14	9	7	0
1972	169	36	25	38	26	20	18	6	0
Tiriyo:									
1966	156	10	23	18	42	33	19	8	3
1970	95	2	11	17	28	18	11	4	2
Xikrin:									
1970	109	21	13	25	31	16	0	1	2
1972	118	30	13	24	35	14	0	1	1
Individual Indians	939	109	132	193	201	145	118	28	13
% Distribution		11.6	14.1	20.6	21.4	15.5	12.6	3.0	1.4
New Haven, Conn., 1957	115	9	25	18	25	13	11	14	
% Distribution		7.8	21.8	15.6	21.8	11.3	9.6	12.2	

crystal violet in 0.1 M citric acid. Plaques were then counted. An 80 per cent reduction was considered significant.

Epstein-Barr virus (EBV). Viral capsid antibody was determined by indirect fluorescent test of Henle and Henle (24), using EB3 cells as the source of antigens. Screening tests were made at 1:5 serum dilution. The results were read independently by two observers. Every fourth specimen was a known positive or known negative control serum.

Hepatitis B. Hepatitis B antigen and antibody testing was initially done with a two-dimensional double immunodiffusion (micro-Ouchterlony) test (25). Where adequate sera were available, confirmatory testing was done with the more sensitive solid phase radioimmunoassay (26). Specificity testing for the radioimmunoassay was conducted by methods outlined by Alter et al. (27). Standard reagent specific sera and subtyping (28) were generously provided by our colleague, Dr. G. L. LeBouvier.

Confirmatory anti-hepatitis B antibody testing was done by the passive hemagglutination technique (29) on all specimens.

Tuberculosis. The standard intradermal Mantoux test was used for tuberculin skin testing, using five units of purified protein derivative. Skin tests were read in 48–72 hours for a positive reaction of 10 mm or more.

Malaria. Spleens were palpated with the subject in a prone position. Blood smears were examined for parasites after staining with Geimsa.

Tetanus. Tetanus toxin neutralization was measured as mouse antitoxic units (30).

RESULTS

General. The serologic data and other information on disease prevalence are summarized in table 3. For convenience, these data have been grouped in classes according to the epidemiologic pattern suggested by distribution of positive test results.

Class I is comprised of those agents to which antibody was found in a high proportion of specimens from every tribe tested. If age differentials in antibody prevalence were found, the differential was confined to the lowest age brackets. This group includes all members of the herpesvirus taxon included in the study. Hepatitis B is also classed with this group, because the proportion of specimens positive was high

TABLE 3

General summary of results: number tested and per cent positive by various tests in separate population groups

Infectious agent	Test*	Minimum positive titer	Tiriyo		Kaxuyana Ewarhoyana				Xikrin		Mekranoti		Kuben KK		Gorotire		All Indian		New Haven, Conn.	
			No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+
Class I																				
Herpes simplex type 1	NT	8	138	91					61	90	116	93					315	95	91	57
Herpes simplex type 2	NT	8	20	90			8	75	60	77	53	85					141	82	91	43
EBV	FA	5	33	97	19	95	9	100	123	97	98	96					282	97		
Cytomegalovirus	CF	4	51	43					29	72							80	54		
Varicella	CF	4	51	41													51	41		
Hepatitis B	Ag RIA		11	0			1	0	132	5	150	11					294	16		
Hepatitis B	Ab HA		11	1			1	0	132	56	150	60					294	40		
Class II																				
Measles	HI	5	154	4	27	89	9	11	106	3	137	0	54	33	120	98	607	28	115	89
Rubella	HI	8	78	95	23	74	9	11	92	0	93	1					295	32		
Mumps	HI	10	184	29	26	73	9	11	103	23	174	22	49	33	120	73	665	33	115	90
Parainfluenza type 1	HI	10	63	9	22	32	9	0	94	20							188	17		
Parainfluenza type 2	HI	10	63	3	22	14	9	0	94	89							188	47		
Parainfluenza type 3	HI	10	63	32	22	78	9	11	94	54							188	47		
Influenza A_0	HI	10	61	2	22	0	9	0	94	0							164	1		
Influenza A_2	HI	10	61	8	22	4	9	0	94	15							164	12		
Influenza B_{GL}	HI	10	63	0	22	0	9	0	94	0							166	0		
Influenza B_{TW}	HI	10	63	0	22	0	9	0	94	0							166	0		
Poliovirus 1	NT	4	147	53					98	30	112	4	45	49			402	33	509	70
Poliovirus 2	NT	4	147	17					98	90	112	96	45	100			402	66	509	65
Poliovirus 3	NT	4	147	40					98	70	112	50	45	47			402	51	509	63
Class III																				
Yellow fever†	HI	10	178	14					102	37	189	5	69	0	219	4	757	11		
Any arbovirus group B	HI	10	178	43					102	39	189	23	69	20	219	23	757	30		
Mayaro†	HI	10	178	47					102	46	189	49	69	20	219	37	757	42		
Any arbovirus group A	HI	10	178	50					102	46	189	49	69	22	219	41	757	44		
Toxoplasmosis	Dye	16	150	43					93	46	112	52					355	47		
Class IV																				
Treponema	FTA; VDRL		29	10	17	0	9	0	85	19	116	54	20	85			276	36		
Tuberculosis	PPD								116	27	185	29					301	28		
Malaria	Spleen								114	41	185	6					299	19		
Tetanus	Mouse AU 400		26	4					51	0							77	1		

* NT, neutralization; FA, fluorescent antibody; CF, complement fixation; Ag RIA, radioimmunoassay antigen; Ab HA, hemagglutination antibody; PPD, purified protein derivative.

† Titer to yellow fever virus or Mayaro was higher than to any other member of their group tested. Yellow fever, Ilhéus, St. Louis Encephalitis and Bussuquara antigens were tested of group B arboviruses and Eastern and Western Equine Encephalitis viruses, Mucambo, Una and Pixuna were tested of group A arboviruses.

relative to that which has been found with the same tests in other population groups and because this high rate occurred in all age categories.

Class II agents include the viruses of measles, rubella, mumps, parainfluenza 1, 2 and 3, influenza A and B, and poliomyelitis. Antibodies to these viruses were either totally missing or had very low frequency in some tribes, but in other tribes showed very high prevalence rates in persons over a specific age. We infer that these are viruses which were not present prior to introduction from larger communities and which spread readily when introduced but which do not persist in these small communities after a sharp epidemic.

The prevalence of antibodies against the arboviruses varied considerably from one tribe to another, but where the overall prevalence was substantial, a clear age gradient was apparent with increasing prevalence in older people. These viruses infect forest animals as well as man, so that in examining the human population, we looked at only one portion of this picture. Isolation from other human populations would not isolate the Indian tribes from these viruses. We surmise from the age pattern of antibody that the viruses had been active in these communities at various times over the years but not generally in the form of intense epidemics. They are identified as Class III. The distribution of Toxoplasma antibody also depended on age and this disease probably also belongs with this class.

In Class IV are agents that cause protracted disease with extended infectious periods. Resolution of epidemic patterns from available data is difficult because the time required for the complete epidemic cycle would be proportionally long. Immunity to tuberculosis and malaria is not sufficiently solid to prevent reinfection and the epidemic patterns would be quite different from those of the virus diseases.

Tetanus is unique in that infection with this agent might involve such a high mortality rate that absence of antibody cannot be taken to indicate that the disease is absent.

In the section that follows, selected data on individual diseases are examined with respect to age and titer distribution.

Class I

Herpes simplex. Herpes simplex type 1 antibody was found in a high proportion of the sera from all tribes tested (figure 2). As noted by Buddingh in his study of New Orleans children (31), few low titers were encountered; thus minor differences in sensitivity of the test have little effect on the proportion recorded positive. The proportion of the Indian sera positive was higher for all age groups in all three major tribes than in our own urban series tested by the same method or in published series tested by different methods (31, 32). Nevertheless, there remained a clear relationship between antibody prevalence and age in children of the Indian tribes. Fifty per cent of the children acquired herpes antibody by two years of age and 75 per cent by four years in the Kayapo tribes. It took a little longer in the Tiriyo.

The distribution of herpes type 2 antibody was closely correlated with that of type 1 both by age (table 4) and by individual specimens. Very few specimens qualified as "type 2" sera by Nahmias' criterion that only sera with higher titer against type 2 than type 1 are clearly indicative of type 2 infection (22). Although most type 2 titers were lower than type 1 in all age groups, the difference was smaller in the older age groups, and many fell into Nahmias' "Intermediate" class (i.e., type 1 titers 0 to 3 times the type 2) (table 4). There was no sharp change in prevalence of these relatively high type 2 antibody titers after puberty, such as might be characteristic of an infection acquired by venereal transmission. It is impossible to say whether this change in

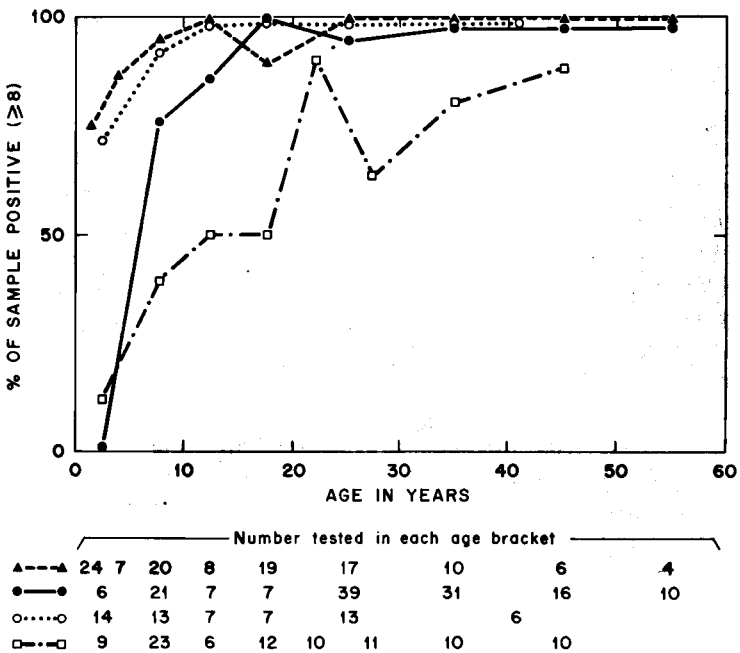

FIGURE 2. Prevalence of neutralizing antibody to herpes simplex type 1 in three Indian tribes and one urban population. In this and subsequent figures, the number of tests incorporated in each point is given in the table below.

TABLE 4

Herpes simplex virus type 2 neutralizing antibody. Number tested and percentage of type 2 titers within 1:3 of type 1 titer

Age	All Indians		New Haven	
	No.	%	No.	%
0–4	30	17	9	0
5–9	27	35	23	0
10–14	9	46	6	0
15–19	14	50	12	0
20–29	21	67	21	4.8
30–39	9	67	10	0
40+	14	36	10	20

relative titer was the result of superinfection with type 2 virus of type 1 positive persons, or if it simply represents a broadening of the type 1 response with prolonged antigenic stimulation. No corresponding broadening was observed in the New Haven population. The number of intermediate titer ratios was low in New Haven, as might be expected from extrapolation from the data on high risk populations studied by Nahmias (22) and by Rawls (33).

EBV. A previous report (15) indicated that 67 per cent of the Tiriyo had EBV antibody. At the time those tests were made, this population had one of the highest proportions of positive sera on record. The sensitivity of the method has been substantially increased in the present study, and 98 per cent of the Tiriyo were found positive. Similar prevalence rates were found in each of the other tribes tested, including the recently contacted Ewarhoyana. The percentage EBV antibody negative was too small to permit analysis of individual tribes by age, and even after data from the five tribes were pooled, it was doubtful whether any evidence of the age of acquisition of immunity could be detected (table 5). Overall, 98.3 per cent of the 291 specimens were recorded as positive, but even under two years of age 95 per cent of 19 sera had

antibody. This remains one of the earliest acquisition rates recorded and is distinctly accelerated relative to the situation in the Connecticut urban community of Danbury (34).

Cytomegalovirus and varicella. The complement fixation tests for cytomegalovirus and varicella do not measure stable antibody (35, 36), so it must be presumed that many persons who were recorded as negative had had experience with the virus in the past.

There was a minor inverse relationship between cytomegalovirus antibody prevalence and age suggestive of waning titers after infection early in life. No correlation could be shown with varicella, where nearly as many children as adults had antibody. The overall antibody prevalence rates are similar to those observed in open populations (37).

Hepatitis B. The results of hepatitis B antigen and antibody testing are shown in table 6. Rates for both hepatitis B antigen and antibody are higher than those reported for industrialized populations, although antigen prevalence does not reach those highest levels previously reported for several Indian and tropical populations (38).

Subtyping of antigen positive sera revealed only the antigen combination "adw." The finding of this antigen subtype among remote populations in both Africa and South America has led LeBouvier to suggest that this may have been the "ancient subtype" (39).

The increasing antibody prevalence with increasing age in children, and the relatively high incidence of antigen carriage in the youngest age group suggest early infection and high endemic rates of hepatitis B in these people. One individual of the Xikrin tribe demonstrated antigen presence on both occasions tested, suggesting the occurrence of chronic carriage and an endemic means of continuance of this infection in these groups.

TABLE 5

Number tested and percentage of sera positive (+) for EBV antibody by fluorescence test

Age (years)	All Indians		Danbury, Conn.	
	No.	%+	No.	%+
<2	19	95	126	11
2-4	37	97	640	19
5-9	37	97	898	32
10-14	21	100	32	57
15-19	39	100		
20-24	24	96	120	86
25-29	36	100	335	88
30-39	42	93	517	91
40-49	23	96	160	96
50+	13	100		

Class II

Measles. No measles antibody was found in any member of the Mekranoti when their tribe was first tested. The one member of the Ewarhoyana who was positive was also uniquely positive for rubella, mumps and parainfluenza antibody, and the only member of the three Carib tribes with Rh negative blood (40). It seems probable that this individual had joined the tribe from some other group and had been exposed to virus infections elsewhere. The three Xikrin who had measles antibody had lived and worked for a time with Neo-Brazilian nut collectors, and the few Tiriyo who had measles antibody were all young adults, the most mobile age group (figure 3). We conclude this pattern indicates that measles virus had never spread within any of these tribes. Infections incurred outside the tribal territory were not carried back with the returning traveler in a state infectious for fellow tribesmen.

The other three tribes tested for measles antibody all had high prevalence rates in the adults and low rates in young children. In Gorotire, specimens were collected 3 years after a measles epidemic, and all but one person over the age of three years had antibody. In Kuben Kran Kegn, 10 years had elapsed since the last recorded epidemic, and 80 per cent of the adults but

TABLE 6

Prevalence of hepatitis B antigen and antibody

	Age (years)						All	%
	0–4	5–9	10–14	15–19	20–29	30+		
Antibody								
Mekranoti	3/23	9/21	5/11	15/26	15/32	12/37	59/150	39
Xikrin								
1970	1/4	2/8	2/6	3/9	4/23	2/13	14/63	22
1972	2/25	4/14	3/9	12/17	10/33	5/17	36/115	31
All* %	12.5	37.5	45.0	67.4	37.7	27.4	102/282	36
Antigen								
Mekranoti	5/23	1/21	0/11	2/26	1/32	2/37	11/150	7.3
Xikrin								
1970	0/4	0/8	0/6	0/10	1/24	2/16	3/68	4.4
1972	0/33	0/14	0/8	0/21	1/30	3/17	4/123	3.3
All* %	8.6	5.3	<5.3	4.3	4.5	9.5	18/291	6.2

* Repeat tests on the same individual counted only once. Includes 12 from Northern tribes.

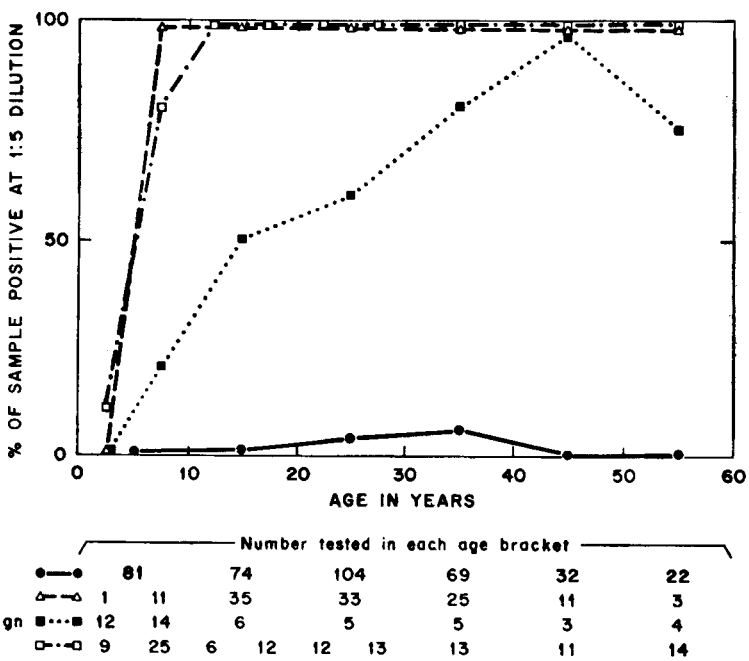

FIGURE 3. Prevalence of measles HI antibody. E, M, X & T represents combined data for tribes with very low prevalence (Ewarhoyana, Mekranoti, Xikrin and Tiriyo, respectively.)

only two children under 10 years old had antibody. In Kaxuyana, no history was available, and everyone over the age of 9 years had measles antibody and no one under 9 years had antibody.

On the basis of the high rates in adult Gorotire and Kaxuyana and by analogy to studies in island populations (7, 41, 42), we believe that the measles epidemics commonly involve essentially everyone in an affected village. The unaffected Kuben Kran Kegn may have been away at the time of epidemic. It is also evident the virus does not commonly persist in a latent

infectious form after an epidemic. Children born later remain without their own antibody to the disease.

Rubella. There was a history of a rubella epidemic among the Tiriyo in 1962 or 1963. Specimens collected in 1966 and 1970 provided serologic confirmation of this epidemic and evidence that the virus was not active in the community after 1963 (table 7). Ninety-three per cent of the specimens from persons born before 1961 had rubella hemagglutination inhibition (HI) antibody, whereas there was only one unconfirmed positive titer in specimens from children born after 1963.

Rubella HI antibody was found in only one member of the Xikrin or Mekranoti tribes.

Poliovirus. The neutralization test for poliomyelitis is not altogether type specific; infection with one type sometimes gives antibody to another type (43). Therefore, some positive reactions are to be expected against types with which there has been no experience. Furthermore, some live vaccine had been distributed among the Tiriyo making antibody prevalence in that tribe irrelevant to the present study.

Type 1 poliovirus neutralizing antibody was found in very few persons of any age in Mekranoti, only in persons over the age of 20 in Xikrin (with two exceptions) and, with one exception, only in persons over 12 years of age in Kuben Kran Kegn (figure 4). This antibody was found in all Kuben Kran Kegn 18 years or older, but in only half the adult Xikrin. Twenty years prior to the date of specimen collections, the Xikrin were living in small scattered groups, whereas the Kuben Kran Kegn were in a consolidated village.

Type 2 poliovirus antibody was very widely distributed in all age groups of the Mekranoti, Xikrin and Kuben Kran Kegn. However, prevalence of this antibody was low in the Tiriyo and what was found may be attributable to vaccine-derived immunity.

Type 3 poliovirus antibody was absent from children under age 5 in Xikrin (with one exception) but was found in older age groups, including 11 of 13 children aged 5 to 9. In Kuben Kran Kegn, the cut-off for type 3 antibody was about 10 years of age. In Mekranoti, it was prevalent in all age groups.

Mumps and parainfluenza. The HI antibodies induced by mumps and by the parainfluenza viruses are neither as specific nor as durable as the antibody systems described above. On several occasions, paired serum specimens showed boosts in titer against one or another of these viruses that were confined to persons who already had detectable antibody (44). Reconstructions of the history of the tribes with respect to these viruses must, therefore, be more limited and more tenuous.

Only two of the Mekranoti, the eldest member of the tribe and one who had traveled most widely, had mumps antibody, and these had only a titer of 1:10, a value which may be nonspecific (45). Titers greater than 1:10 in the Tiriyo collection were confined to sera from persons over 17 years old, and in the Xikrin were limited to persons over 6 (figure 5). However, even over these ages, the proportion positive and the average titer of positive

TABLE 7

Number tested and percentage of sera positive (+) for rubella HI antibody

Age	Danbury, Conn.*		Tiriyo†		Xikrin and Mekranoti	
	No.	%+	No.	%+	No.	%+
1–4						
3	378	26.2	17	29	16	0
3–4	488	32.0				
5–9						
5–6	488	37.3	19	79	17	0
7–8	379	46.2				
10–14	80	68	8	100	18	0
15–19	17	71	8	100	35	3
20–29	160	73	46	98	51	0
30–39	39	69	26	92	24	0
40–49			14	93	11	0
50+			8	88	10	0

* Data from McCollum et al. (64).

† Tiriyo ages as of 1966, three years after reported rubella epidemic.

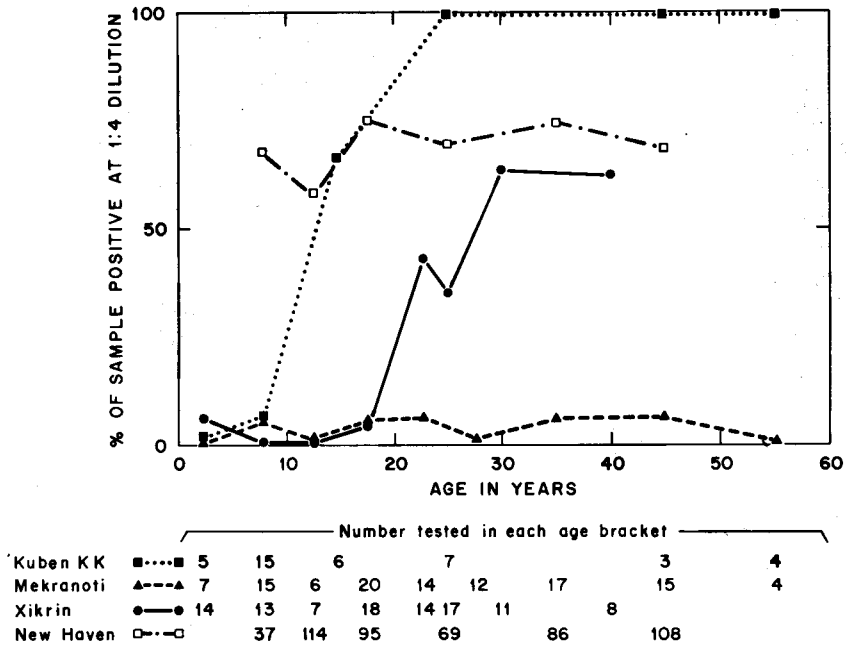

FIGURE 4. Prevalence of poliovirus type 1 neutralizing antibody.

specimens was low relative to that found in New Haven. The number of specimens from Gorotire children under age 5 was too small to give meaningful data, but the distribution of positive tests and titer in older Gorotire was indistinguishable from that in New Haven. Most Kuben Kran Kegn adults had mumps antibody in moderate titer and most children did not.

Parainfluenza 1 antibody was found in very few Tiriyo of any age and none under 20 (figure 6). In the Xikrin, too, there was no evidence of parainfluenza 1 under age 17, but it was commonly found in persons of 30 to 40 years.

Parainfluenza 2 was frequently found in Xikrin of all ages, but was almost entirely absent from the northern tribes (figure 7). Parainfluenza 3 was present in most Kaxuyana sera but relatively few Tiriyo or Xikrin. In the latter tribes, positive results were found at all ages, but high titers were almost entirely confined to specimens from adults.

It is not possible to state with any assurance when these viruses were last active in each of these tribes, but it is clear that the prevalence of antibody is unusually low in all of them. In contrast to these results, Parrott et al. (46) found that most children in their Washington, D.C., study had neutralizing antibody against these viruses by their fourth birthday, and Taylor-Robinson (47) reported 100 per cent of adult sera from various countries positive for all three viruses by the HI test.

Influenza. Very few persons had antibody to any of the influenza antigens used in our tests. No one had influenza B antibody. The fact that influenza A_2 was slightly more common than A_0 confirms the supposition that the tribes have been less isolated since 1957 than they were earlier. These results echo similar findings by Neel et al. in the Xavante Indians of the Mato Grosso who were devoid of influenza A_2 antibody and had a very low prevalence of influenza B antibody (14).

Smallpox. No pock scars indicative of variola were seen in any member of any

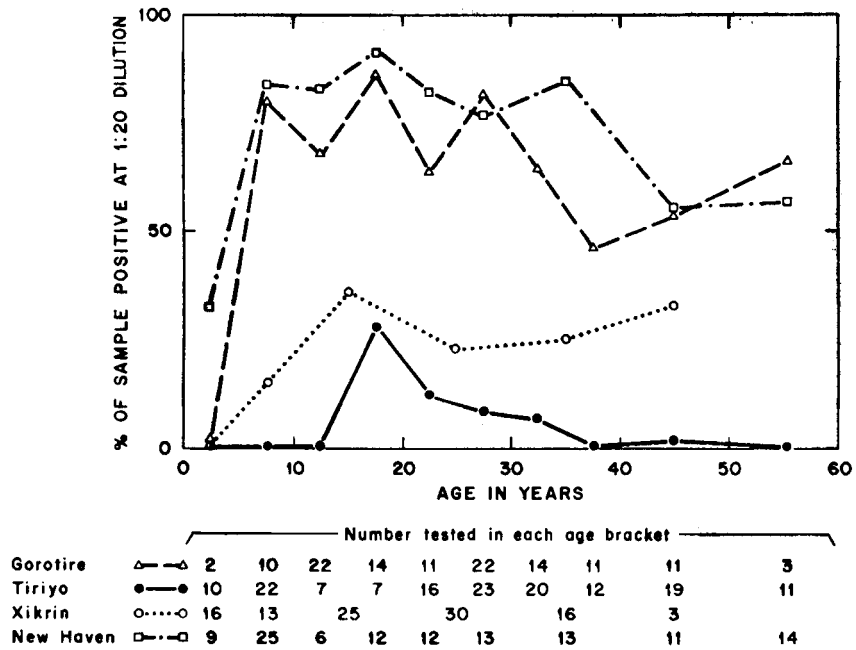

FIGURE 5. Prevalence of mumps HI antibody.

tribe. Vaccinia had not been administered to the Northern tribes, the Xikrin or the Mekranoti.

Class III

Group B arbovirus. Yellow fever virus accounted for most of the Group B arbovirus antibody in Tiriyo and Xikrin, although broad group reactions were commonly found. Ilhéus HI antibody was sometimes found without antibody to other members of the group and may have been chiefly responsible, with yellow fever for the group-specific reactions characteristic of multiple infections. Ilhéus was the most commonly encountered member of the group in Kuben Kran Kegn, Gorotire and Mekranoti. Yellow fever HI antibodies were also found in a small proportion of the latter two tribes, and the specificity of these reactions was confirmed by neutralization tests. There were no yellow fever positive specimens from Kuben Kran Kegn. Tests for antibody to St. Louis encephalitis and Bussaquera were also carried out, but specific reactions were not observed.

Antibody to yellow fever is known to be maintained for many years without reexposure (48) and, hence, it is reasonable to assume that the patterns observed represent cumulative life-time histories. There was a steady progression toward increasing antibody prevalence with increasing age in each of the tribes studied (figure 8). There was no evidence of a sudden increase in prevalence at any one age bracket that might indicate a major epidemic in the past. The curves suggest that these viruses are endemic as a group and that the chances that any one person has encountered them are proportional to his life span.

Arbovirus group A. Mayaro antibodies were the most frequently encountered of the group A arbovirus HI antibodies in all tribes studied. There was some broadening of the response to include other members of the group in Tiriyo. This seemed to be attributable to activity of some member of

the Venezuelan equine encephalitis virus subgroup (15).

There is less information on the durability of antibody titer against these viruses than against the group B arboviruses. Except in the Tiriyo, where prevalence of group A arbovirus antibody reached very high levels in the older adults, there seemed to be leveling of antibody prevalence after childhood (figure 9). This may indicate that these viruses were not active in the area more than 20 years ago, but it may be antibody levels tend to fall below the measurable threshold some years after initial infection in the areas where only one member of the virus group is active. In children, the incidence of positive specimens increased with age in a regular manner, suggesting virus endemicity in recent years.

Toxoplasma. Antibodies to *Toxoplasma gondii* were absent from children under 5 years of age in the Tiriyo and under 15 in the Mekranoti and Xikrin but they were found in adults with increasing frequency as age increased. This pattern contrasts with that found by Neel et al. (14) in the Xavante where toxoplasma antibody was prevalent in all age groups, and with that found by Wallace et al. (65) in New Guinea where antibodies were found only if domestic cats were present. There are no domestic cats in the tribes we studied but various wild cats are hunted for prestige and food. Insofar as we could determine, the only point at which adults of both sexes might be exposed to uncooked cat products is in the process of butchering.

Class IV

Treponema. Only three of 50 members of the northern tribes gave sera that were positive by both Venereal Disease Research Laboratory (VDRL) test and the fluorescent treponemal antibody absorption FTA-ABS test. These three persons were all adult; two were women.

In contrast, the prevalence of positive

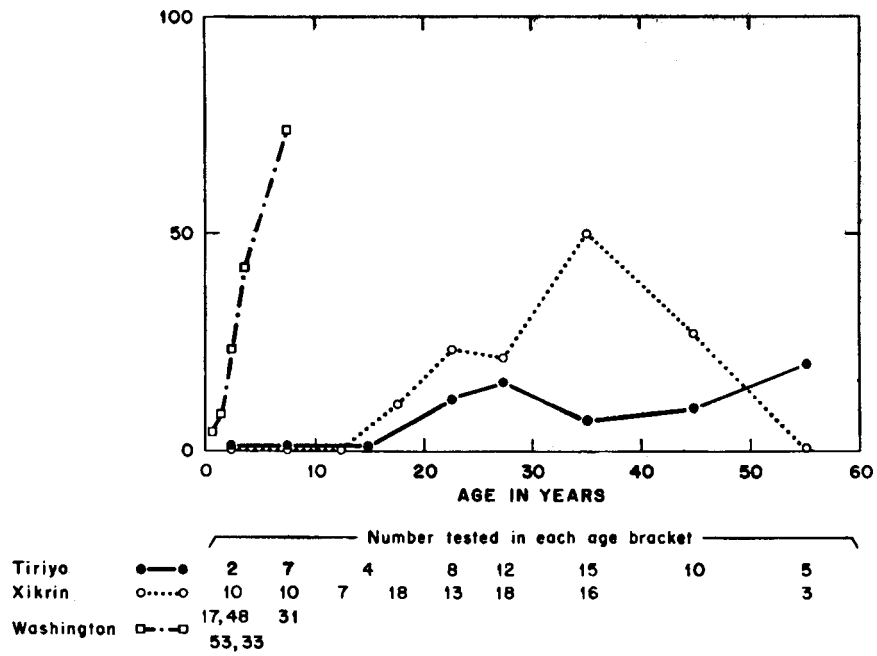

FIGURE 6. Prevalence of parainfluenza type 1 HI antibody. Data from Parrott et al. (46) for children in Washington, D.C., are included for comparison.

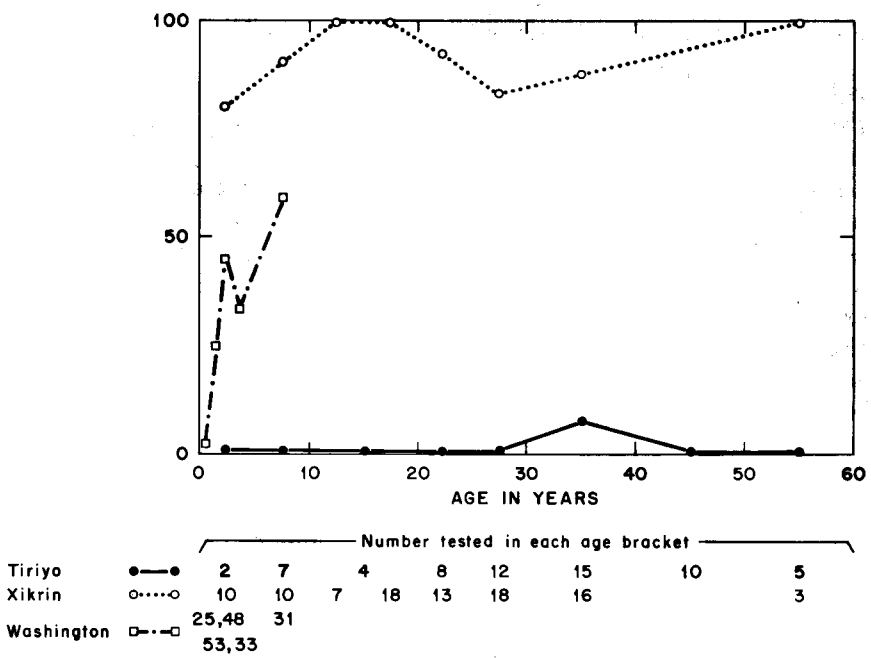

FIGURE 7. Prevalence of parainfluenza type 2 HI antibody.

sera in the southern tribes was very high (figure 10). In the Xikrin, 35 per cent, in the Mekranoti 54 per cent, and in the Kuben Kran Kegn 84 per cent of the adults were positive by both tests. In addition to the specimens that were positive for both tests, 6.9 per cent of the specimens from these three tribes were positive by VDRL only and 12.2 per cent by FTA only. The VDRL "false positive" tests were usually low in titer and they were found most frequently in young children. Malaria may have caused the false positives, although the rate of spleen enlargement was not unusually high in false positive subjects. The larger proportion positive by FTA only were distributed by age in a pattern similar to reactions by both tests.

The prevalence of treponemal antibody in children of the southern tribes was much lower than in adults, although positive reactions by the two tests were noted in children as young as 9 years of age. There was no correlation of test results between spouses. Of 27 couples for whom full data are available, both partners were positive in 11 instances, but they differed from each other in 14 instances. Both were negative in two instances. Sixteen of 32 offspring of positive mothers were themselves positive, whereas only 6 of 21 offspring of negative mothers were positive for both tests. However, there was a strong age correlation within the offspring groups, suggesting that the antibody was acquired years after birth. The familial correlation could not be confirmed by comparison of sibs. Physical examination revealed no evidence of congenital syphilis or pinta, and only one lesion that might have been caused either by yaws or by leishmaniasis.

Malaria. Examinations for splenomegaly were carried out on members of the Xikrin and Mekranoti at the end of the rainy season and blood smears were prepared at the same time. Parasite positive smears were found only in young Xikrin, and the four infections encountered were all *Plasmodium falciparum* with scant numbers of gametocytes. None of these four children had enlarged spleens. However, 38 per cent of the Xikrin had palpable spleens. Twenty

of these spleens were characterized as large, 12 being at or below the umbilicus. Among the Mekranoti, the spleen rates were lower and the sizes smaller (table 8).

In the absence of definitive information about possible malaria control activities, including both insecticide spraying and antimalarial drug administration, it is not possible to identify a specific level of endemicity as outlined by the WHO technical committee (49). Antimalarials, including chloroquine-treated salt, have been widely distributed in poorly controlled but nonetheless significant volume.

The detection of parasites only in the young, and only gametocytes, coupled with a significant number of large spleens, suggests a high degree of malaria endemicity in the Xikrin. The position of the Mekranoti is less clear, but malaria, presumably present, would appear to be at a lower level of endemicity.

According to the WHO classification (49), these spleen rates indicate that malaria is hypoendemic in Mekranoti and mesoendemic in Xikrin. However, this classification presumes the acquisition of immunity by adults, and the ability of the Southern American Indian to develop sufficient immunity to prevent splenomegaly is unproved. Neel (14) has suggested that spleen enlargement rates, such as those of the Xikrin which Schaad (50) also found in Indians of Surinam, may represent a much greater burden to these people than similar rates in races who have more resistance to the disease.

Tuberculosis. Tuberculosis probably represents the most serious immediate threat to the survival of the Indian tribes. The increasing prevalence of positive tuberculin reactions and the fact that the highest rates are found in the teen-age group (table 9) suggest that this is an infection which has not yet established an equilibrium with its host. Nutels has discussed this problem in relation to the Indians of the Xingu Park (51, 52), where he also found a rapidly increasing proportion of tuberculin reactors in serial tests.

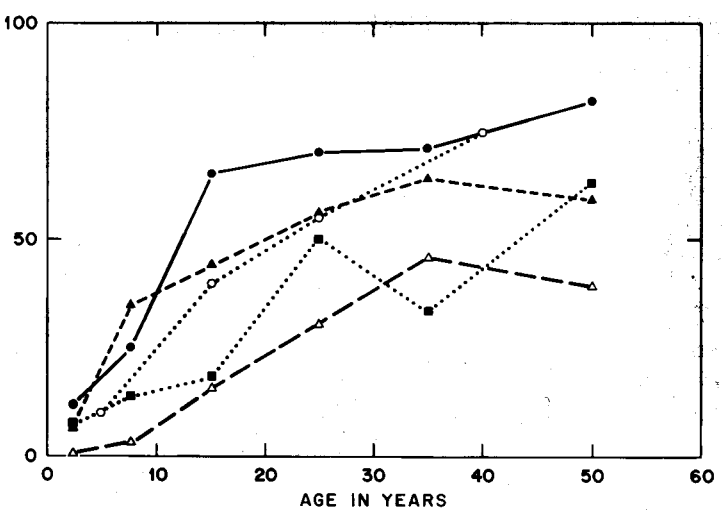

FIGURE 8. Prevalence of HI antibody to yellow fever or Ilhéus virus.

Tetanus. Antibody against tetanus, as measured by toxin neutralization in mice, was only found at a low level (0.0025 units) in one out of 77 persons in two tribes tested.

Filiariasis. The blood smears examined for plasmodia were also scanned for filariasis. None was found. This is in accordance with negative findings by Schaad in Surinam (50) and Neel in the Xavante (14). Approximately one third of 187 specimens examined were collected after dark, when *Wuchereria bancrofti*, if present, might have been active.

General discussion

Populations as primitive and isolated as those studied in this report are fast disappearing and their health status might be considered of little lasting consequence. However, until 7000 years ago all mankind lived in settings sociologically much more like those of these modern primitive survivors than like any cosmopolitan community. Efforts to reconstruct the environment in which man and his pathogens evolved have been largely dependent on bony remains (53), but these shed light on few infectious diseases. Diseases of soft tissues have been studied in Egyptian mummies, but these came from a relatively advanced cultural level. Polunin has considered contemporary primitive societies in Malaysia as a model for the conditions of antiquity (54). He has emphasized the importance of isolation of small groups in determining disease patterns, but the populations he studied were less isolated than those considered in the present report.

The fact that many common diseases had been absent for very long periods of time from the tribes we have studied does by itself prove that the agents causing them could not have persisted in these cultures. The absence of any evidence of smallpox, for instance, may mean that the virus evolved since the dispersal of these people from the old world population centers and that it has yet to reach these tribes. Another possibility to be considered is a slow movement of these viruses in a narrow stream over a large area so that intervals approximating a life span might occur between epidemics in any one tribe. Measles has been observed to spread from

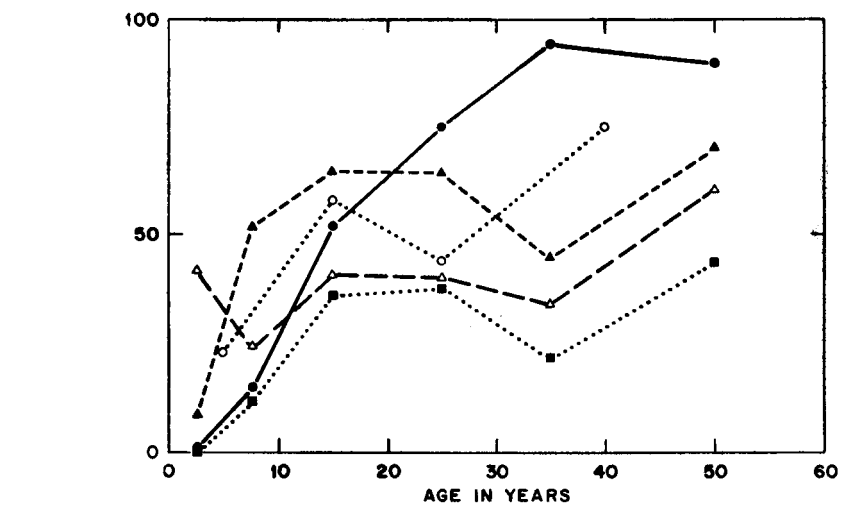

FIGURE 9. Prevalence of HI antibody to Mayaro or Pixuna.

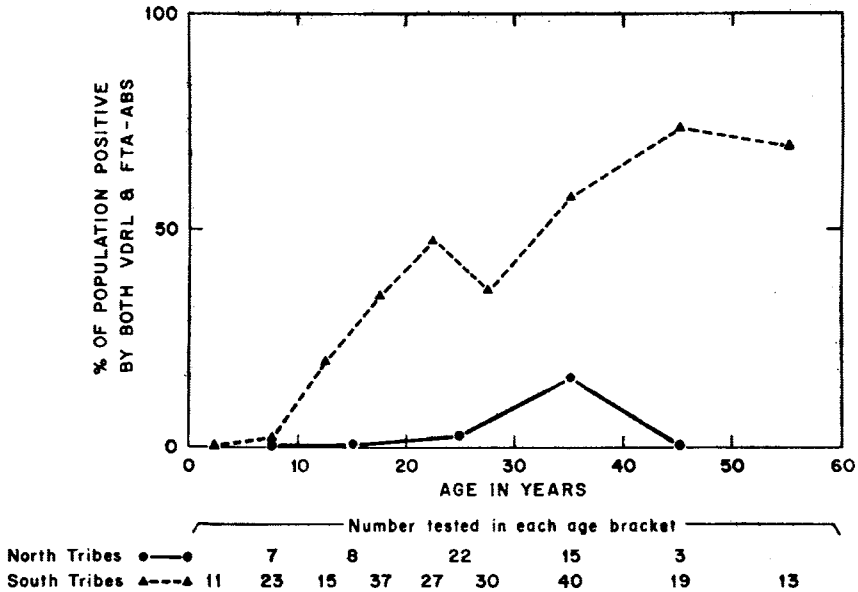

FIGURE 10. Prevalence of sera reactive by both VDRL and FTA-ABS tests for treponema infection. South tribes included Kuben Kran Kegn, Mekranoti and Xikrin.

TABLE 8

Evidence of malaria

Age	Xikrin		Mekranoti	
	Spleen palpable	Parasit-emia	Spleen palpable	Parasit-emia
>2	3/18	2/25	0/20	0/16
2–4	2/13	1/11	0/27	0/24
5–9	4/12	1/11	2/25	0/21
10–14	5/7	0/8	2/12	0/4
15–19	9/17	0/19	2/27	0/1
20–30	17/30	0/28	3/31	0/1
30–40	3/15	0/13	1/21	0/2
40+	1/3	0/3	1/23	
Total	44/115	4/118	11/186	0/69

TABLE 9

Number tested and percentage positive (+): tuberculin reactions of more than 10 mm in duration

Age in 1972	Xikrin 1970		Xikrin 1972		Merkranoti 1972	
	No.	%+	No.	%+	No.	%+
>2			18	0	21	14
2–4	26	3.9	25	8	29	38
5–9	16	19	14	21	25	56
10–14	9	45	9	55	12	84
15–19	15	67	18	72	26	77
20–24	13	46	17	65	17	77
25–29	15	40	18	61	15	73
30's	12	50	14	57	23	83
40's					17	59
50+	3	33	3	33	6	50
All ages	109	34	136	41	191	59

village to village, where neighboring groups have been "pacified" by Neo-Brazilians. Prior to pacification, however, peaceful contacts between tribes seem to have been very limited. Raids to capture children or wives were not uncommon, as evidenced by the number of persons who had entered each tribe in this way. The captives would be more likely to introduce chronic infections like tuberculosis than diseases with a short infectious period like measles.

Our findings in these tribes regarding the specific agents which persist are very similar to the hypotheses put forward by Hare (55). He has set up postulates as to what diseases probably occurred in ancient peoples based on epidemiologic considerations and historic records. There are a few specific differences. Hare grouped herpes-zoster with the diseases of recent origin. We found it endemic in our tribes. Conversely Hare grouped poliomyelitis with the diseases capable of surviving in a paleolithic society.

The agents that failed to persist in these populations included all those viruses included in the study which are known to have infectious periods lasting only a few

days. In addition, poliovirus, which may be excreted for several months after infection (56), failed to persist. There has been earlier evidence that poliovirus dies out of small populations after an epidemic (57). Rubella may be excreted by infants up to two years after infection in utero (58), but this was apparently not sufficient to insure its persistence. The initial rubella epidemic in Tiriyo conferred immunity on a very large proportion of the population so that the opportunity for a series of in utero infections would be negligible.

The failure to find tetanus neutralizing antibody in the Tiriyo and Xikrin serum specimens is not surprising since immunization had not been practiced in these tribes except possibly in an occasional individual, and also since it is generally believed that antibodies do not arise as a result of natural infection. To be sure, two recent studies (59, 60) have reported finding a high incidence of tetanus antitoxic antibodies in persons who were considered to have had no opportunity or occasion to be immunized. Veronesi et al. (60) determined by "insistent" questioning that his subjects had not received tetanus vaccine, but some doubt must always remain in an area where vaccination is common practice. The present results indicate that our populations have not been vaccinated and that either the organism is not present in the villages, or that in the natural course of events antibodies are not induced to measurable titer.

The viral agents that were found to survive in the Indian tribes were confined to those that are known to persist in the body for very long periods after initial infection. Herpes simplex and varicella are known to be periodically reactivated, and the secondary forms of the disease are infectious. There is evidence to indicate cytomegalovirus reactivation during pregnancy (61), and the same might be true of EBV if appropriate techniques were available to demonstrate it. A hepatitis B antigen carrier state lasting at least 2 years was observed in the Xikrin. It is, therefore, not surprising that all of these disease agents could maintain themselves in a population of a few hundred persons. The very high level of their endemicity is striking, however. Apparently, the disease agents spread very effectively in these closely knit communities.

The pattern of treponemal antibody which was observed in the southern tribes is most unusual. The sexual mores of these tribes are more permissive than those of the north, yet monogamy is the rule. The total lack of any correlation between the reactions observed in spouses suggest that the agent was not venereally spread. The weakness of the mother-offspring correlation is also at variance with the usual pattern for syphilis. The high proportion of positive FTA tests where the VDRL was negative suggests that some treponema other than *T. pallidum* may have been involved. Pinta has been reported to occur in Kuben Kran Kegn (62); the same study reported it absent from Gorotire and Xikrin. We found no evidence of it on detailed examination of nearly every member of the Xikrin and Mekranoti nor on cursory observation of 75 Kuben Kran Kegn. Whatever the nature of the agent causing these reactions, it seemed to be highly adapted to this culture.

Malaria and tuberculosis contrast with the endemic virus and treponemal diseases in their high morbidity. The increasing prevalence of positive tuberculin reactions in this and in Nutels' study (51) suggests that tuberculosis may have been recently introduced. We have no similar evidence with respect to malaria, but the human genes which are thought to confer resistance to malaria are all absent from these populations (40, 63). Various authors have speculated that malaria was introduced to the New from the Old World (53). Neither malaria nor tuberculosis in these villages seem to have established the relationship with their hosts that would provide optimal opportunity for survival of the agent.

References

1. Panum PL: Observations Made during the Epidemic of Measles on the Faroe Islands in the Year 1846. New York, American Publishing Association, 1940
2. Paul JH, Freese HL: An epidemiological and bacteriological study of "common cold" in an isolated Arctic community (Spitzbergen) Am J Hyg 17:517–535, 1933
3. Hope Simpson RE: Studies on shingles. Is the virus ordinary chickenpox? Lancet 2:1299–1302, 1954
4. Bartlett MS: The critical community size for measles in the United States. J Roy Stat Soc Ser A 123:37–44, 1960
5. Black FL: Measles endemicity in insular populations. Critical community size and its evolutionary implication. J Theor Biol 11:207–211, 1966
6. Rosen L, Thooris C: Poliomyelitis in French Oceania. Epidemiologic observations on an outbreak with notes on the incidence of paralysis following intramuscular injections. Am J Hyg 57:237–252, 1953
7. Adels NR, Francis JW, Gajdusek DC: Measles in Australasian indigenes. Am J Dis Child 103:255–260, 1962
8. Brown P, Gajdusek DC, Morris JA: Epidemic A_2 influenza in isolated Pacific island populations without pre-epidemic antibody to influenza virus types A and B, and discovery of other still unexposed populations. Am J Epidemiol 83:176–188, 1966
9. Tyrell DAJ, Peto M, King N: Serologic studies on infections by respiratory viruses of the inhabitants of Tristan da Cunha. J Hyg (Camb) 65:327–341, 1967
10. Illman D, Mufson MA: Viral antibodies in the Korekore tribe of Northern Rhodesia. Trop Geogr Med 23:64–70, 1971
11. Anderson N, Mufson MA: Viral antibodies among the Turkana people of Northern Kenya. Trop Geogr Med 24:168–177, 1972
12. Van Reken D, Mufson MA: Viral antibodies in the Mano tribe of Northern Liberia. Trop Geogr Med 24:303–310, 1972
13. Brown PK, Taylor-Robinson D: Respiratory virus antibodies in sera of persons living in isolated communities. Bull WHO 34:895–900, 1966
14. Neel JV, Salzano FM, Juqueira PC, et al: Studies on the Xavante Indians of the Brazilian Mato Grosso. Hum Genet 16:52–140, 1964
15. Black FL, Woodall JP, Evans AS, et al: Prevalence of antibody against viruses in the Tiriyo, an isolated Amazon tribe. Am J Epidemiol 91:430–438, 1970
16. Frikel P: Os Kaxuyana Nota etnohistoricas. Museu Paraense E Goeldi Belem Publ Avulsas 14:1–82, 1972
17. Diniz ES: Os Kayapo-Gorotire. Aspectos socioculturais do momento actual. Bol Mus E Goeldi Belém 18NS:1–40, 1962
18. Rocha FJ da, Salzano FM: Anthropometric studies in Brazilian Kayapo Indians. Am J Phys Anthropol 36:95–101, 1972
19. Frikel P: Fases culturais e aculturacão intertribal no tumucumaque. Bol Mus E Goeldi Belém 16NS:1–90, 1961
20. Frikel P: Notas sobre a situacão actual dos indios Xikrin do rio Caetete. Rev Mus Paulista São Paulo 14NS:145–158, 1963
21. Niederman JC, Horstmann DM, Opton EM: A survey of poliovirus antibody levels in New Haven, Connecticut, 1957–58. Am J Hyg 72:218–230, 1960
22. Nahmias AJ, Josey WE, Naib ZM, et al: Antibodies to herpesvirus hominis types 1 and 2 in humans. I. Patients with genital herpes infections. Am J Epidemiol 91:539–546, 1970
23. Gudnadottir M, Helgadottir H, Bjarnason D, et al: Virus isolated from the brain of a patient with multiple sclerosis. Exp Neurol 9:85–95, 1964
24. Henle G, Henle W: Immunofluorescence in cells derived from Burkitt's lymphoma. J Bacteriol 91:1248–1256, 1966
25. LeBouvier GL, McCollum RW: Australia (hepatitis associated) antigen: physicochemical and immunological characteristics. Adv Virus Dis 16:357–396, 1970
26. Ling CM, Overby LR: Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with ^{125}I-antibody. J Immunol 109:834–841, 1972
27. Alter HJ, Holland PV, Purcell RH, et al: The Ausria test: critical evaluation of sensitivity and specificity. Blood 42:947–957, 1973
28. LeBouvier GL: The heterogeneity of Australia antigen. J Infect Dis 123:671–675, 1971
29. Vyas GN, Shulman NR: Hemagglutination assay for antigen and antibody associated with viral hepatitis. Science 170:332–333, 1970
30. Clenny AT, Stevens MF: Laboratory control of tetanus prophylaxis. J Roy Army Med Corps 70:308–310, 1938
31. Buddingh GJ, Schrum DI, Lanier JC, et al: Studies of the natural history of herpes simplex infection. Pediatrics 11:595–610, 1953
32. Yoshino K, Kaniguchi S, Furuse R, et al: A serological survey for antibodies against herpes simplex virus with special reference to comparatively heat-labile complement-fixing antibodies. Jap J Med Sci Biol 15:235–247, 1970
33. Rawls WE, Gardner HL, Flanders RW, et al: Genital herpes in two social groups. Am J Obstet Gynecol 100:682–689, 1971
34. Nimminitra S: A survey of EBV antibodies among healthy children. Thesis for MPH degree, Yale Univ School of Med, 1969
35. Weller TH, Witton HM: The etiologic agents of

varicella and herpes zoster. Serologic studies with the viruses as propagated in vitro. J Exp Med 108:869–890, 1958
36. Starr JG, Calafiore, D, Casey HL: Experience with a human cytomegalovirus complement-fixing antigen. Am J Epidemiol 86:507–512, 1967
37. Wentworth BB, Alexander ER: Seroepidemiology of infections due to members of herpesvirus group. Am J Epidemiol 94:496–507, 1971
38. Blumberg BS, Sutnick AI, London WT: Hepatitis and leukemia: their relation to Australia antigen. Bull NY Acad Med 44:1566–1586, 1968
39. LeBouvier G: The subtypes of hepatitis B antigen. Ann Int Med 79:894–896, 1973
40. Salzano FM, Woodall JP, Black FL, et al: Blood groups, serum proteins and hemoglobins of Brazilian Tiriyo Indians. Hum Biol (in press)
41. Christensen PE, Schmidt H, Bang HO, et al: An epidemic of measles in southern Greenland. Measles in virgin soil. II. The epidemic proper. Acta Med Scand 144:430–449, 1952
42. Black FL, Rosen L: Patterns of measles antibodies in residents of Tahiti and their stability in the absence of reexposure. J Immunol 88:725–731, 1962
43. Melnick JL, Ramos-Alvarez M, Black FL, et al: Poliomyelitis viruses in tissue culture. VII. Experiences with viral and serological diagnostic procedures. Yale J Biol Med 26:465–485, 1954
44. Black FL, Woodall JP, Pinheiro FP: Measles vaccine reactions in a virgin population. Am J Epidemiol 89:168–175, 1969
45. Black FL, Houghton WJ: The significance of mumps hemagglutinin inhibition titers in normal populations. Am J Epidemiol 85:101–107, 1967
46. Parrott RH, Vargosko AJ, Kim HW, et al: Acute respiratory diseases of viral etiology. III. Myxoviruses: parainfluenza. Am J Public Health 52:907–917, 1962
47. Taylor-Robinson D: Respiratory virus antibodies in human serum from different regions of the world. Bull WHO 32:833–847, 1965
48. Downs WC, Anderson CR, Delpeche KA, et al: Arthropod-borne encephalitis virus in the West Indies area. II. A serological survey of Barbados. West Indian Med J 11:117–122, 1962
49. Covell G, Russell PF, Swellengrebel NH: Malaria terminology. WHO Monograph No 13:1953
50. Schaad JDG: Epidemiological observations in bush Negroes and Amerindians in Surinam. Trop Geogr Med 12:38–46, 1960
51. Nutels N: Medical problems of newly contacted Indian groups. PAHO Sci Publ 165:68–76, 1968
52. Nutels N, Ayres M, Salzano FM: Tuberculin reactions, x-ray and bacteriological studies in the Kayapo Indians of Brazil. Tubercle 48:195–200, 1967
53. Janssens PA: Paleopathology, Diseases and Injuries of Prehistoric man. London, John Baker, 1970
54. Polunin IV: Health and disease in contemporary primitive societies. *In* Diseases in Antiquity. Edited by D Brothwell and AT Sandison. Springfield, Ill, Charles C Thomas, 1967, pp 69–97
55. Hare R: The antiquity of diseases caused by bacteria and viruses, a review of the problem from the bacteriologist's point of view. *In* Diseases in Antiquity. Edited by D Brothwell and AT Sandison. Springfield, Ill, Charles C Thomas, 1967, pp 115–131
56. Paul JR: The epidemiology of poliomyelitis. WHO Monograph No 26:1955
57. Paul JR, Riordan JT, Melnick JL: Antibodies to three different antigenic types of poliomyelitis virus in serum from North Alaskan Eskimos. Am J Hyg 54:275–285, 1951
58. Phillips CA, Melnick JL, Yow MD, et al: Persistence of virus in infants with congenital rubella and in normal infants with a history of maternal rubella. JAMA 193:1027–1029, 1965
59. Vakil BJ, Tulpule TH: A comparison of 1500 units and 5000 units of tetanus antitoxin in simultaneous active and passive immunization against tetanus. Indian J Med Res 56:1–14, 1968.
60. Veronesi R, Cecin H, Correa A, et al: New approaches on tetanus immunization: Naturally acquired immunity. Preliminary report. Rev Hosp Clin 28:313–318, 1973
61. Reynolds DW, Stagno S, Hosty TS, et al: Maternal cytomegalovirus excretion and perinatal infections. N Engl J Med 285:1–5, 1973
62. Bellizzi AM: Alguns dados sobre a incidencia nosologica nos indios Kayapo. J Brazil Med 5:197–211, 1961
63. Salzano FM, Neel JV, Weitkamp LR, et al: Serum proteins, hemoglobins and erythrocyte enzymes of Brazilian Kayapo Indians. Hum Biol 44:443–458, 1972
64. McCollum RW, Randolf MF, Byrne EB, et al: Antigenic and protective efficacy of live attenuated rubella virus vaccine (HPV 77-DE 5) Am J Dis Child 118:186–189, 1969
65. Wallace GD, Zigas V, Gajdusek DC: Toxoplasmosis and cats in New Guinea. Am J Trop Med Hyg 23:8–14, 1974
66. Black FL, Hierholzer WJ, Woodall P, et al: Intensified reactions to measles vaccine in unexposed populations of American Indians. J Infect Dis 124:306–317, 1971