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[I-11] PRE-EPIDEMIC STATUS OF CHOLERA IMMUNITY AMONG THE GENERAL POPULATION OF GHANA

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INTRODUCTION

In August 1970, a cholera epidemic broke out in Guinea, West Africa, where it had never been noted before. In September 1970, the first case of cholera ever identified in Ghana was notified which was an imported case from Guinea[1]. After a while, the cholera epidemic spread predominantly over the southern coastal regions of Ghana[2]. The number of reported cases reached as many as 8,234 with 417 deaths by the end of March 1971[3].

It is an interesting subject to investigate the immunity status of cholera among the general population of Ghana before the epidemic. Therefore, a serological investigation was made with sera which had been collected for other purposes before the first case of cholera had been found. The present report is to describe the results obtained with these sera.

MATERIALS AND METHODS

1. *Serum Samples.*

Five hundred and seventy-five serum samples collected from three regions of Ghana for various purposes as shown in Table 1 were used. These sera were collected from December 1969 to July 1970 and kept frozen at −20°C.

Sera taken from persons at pre- and post-vaccination times were used as negative and positive controls in the test. The post-vaccination sera taken 14 days after a single injection of 0.5 ml dose of cholera vaccine gave titers of 1:32 for the vaccine antigen and 1:64 for the isolated cholera antigen, whereas the pre-vaccination sera were negative for both antigens at the lowest serum dilution of 1:2.

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TABLE 1
Serum specimens

Region	Number of specimen	Time of collection	Note
Greater Accra	230	March to July, 1970	Korle Bu, Accra. (Kahn test, pre-vaccination serum against yellow fever, hepatitis and jaundice cases)
Eastern	108	April to July, 1970	Akwatia and Akuse (Pre-vaccination serum against yellow fever, jaundice and hepatitis cases)
Upper	237	December, 1969 to January, 1970	Sandema Hamile, Paga, Kokogu, Jirapa, Nandom, Lawra and Bolgatanga. (Pre-vaccination serum against yellow fever)
Total	575	December, 1969 to July, 1970	

2. *Antigen used.*

A suspension of heat killed *Vibrio cholerae*, biotype Eltor, serotype Ogawa (C/00 strain Accra 1), was mainly used as the antigen for the test. The cell suspension was supplied by Dr. C.A. Salles, Reference Laboratory, Ministry of Health, Accra, Ghana, who isolated this strain from the first case of cholera in Ghana, 1970[1]. The vibrio was grown on a nutrient agar slant at 37°C for 18 hours. The cultured cells were harvested and suspended in 5 ml of phosphate buffered saline, pH 7.4, followed by killing at 60°C for 20 minutes. The optical density of the heat-killed suspension was adjusted to 2.5 with a filter of 622 μ using the EEL electrophotometer. This original suspension was diluted 10 times with physiological saline before used as antigen.

Cholera vaccine (Lot No. 42133) produced by Swiss Serum and Vaccine Institute, Berne, containing 8,000 million of *V. cholerae* (Inaba, Ogawa and Eltor) was used also as antigen in some comparative experiments.

3. *Agglutination test.*

Micromethod described by Benenson *et al.*[4] was applied to the test using U shaped plates. For the convenience of reading the agglutinating patterns on microplates, 40 parts of antigen suspension were mixed for staining with 1 part of diluted carbol fuchsin solution of common bacteriological use. To 0.025 ml of serial dilution of serum in physiological saline was added 0.025 ml of fuchsin stained antigen. The serum-antigen mixtures were incubated at 37°C for 1 hour and then kept at 4°C overnight before reading with the naked eye. Antigen and serum controls with positive and negative reactions were set up at the same time.

TABLE 2
Distribution of cholera agglutinating antibody titer in Ghana specified by regions

Region	Number tested	Number negative	Number positive at a titer of					
			1:2	1:4	1:8	1:16	1:32	1:64
Greater Accra	230	171 (74)*	28 (12)	16 (7)	9 (4)	4 (2)	20 (1)	0
Eastern	108	51 (47)	17 (16)	21 (20)	11 (10)	6 (6)	1 (1)	7 (1)
Upper	239	171 (72)	40 (17)	17 (7)	4 (2)	4 (2)	0	1 (0.4)
Whole Ghana	575	393 (68)	85 (15)	54 (9)	24 (4)	14 (2.4)	3 (0.5)	2 (0.3)

* Figure in brackets indicates percentage.

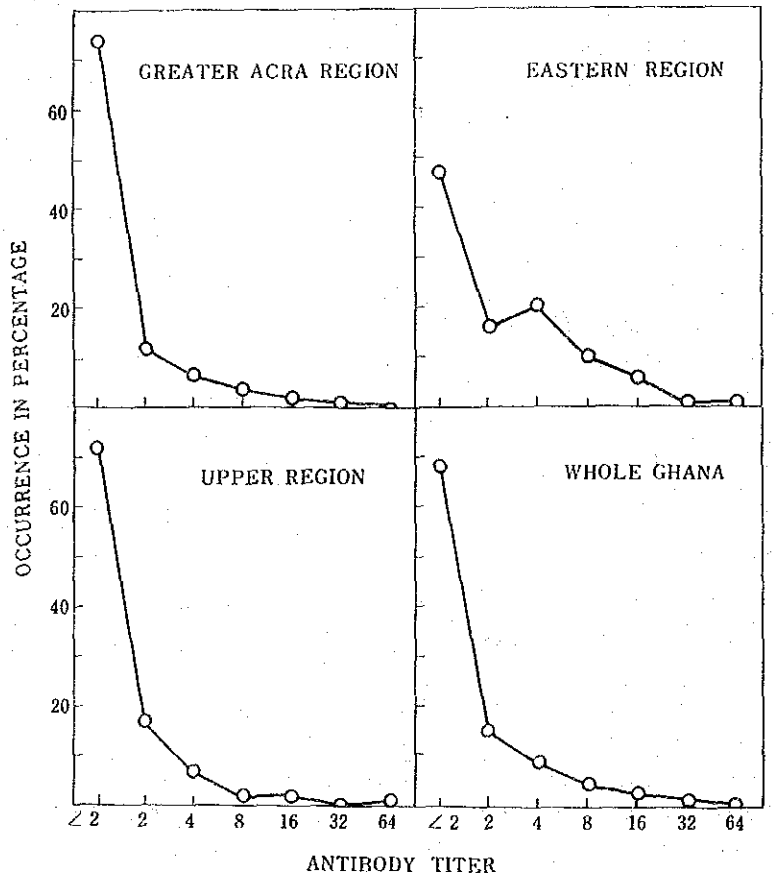

FIG. 1. Titer distribution of cholera agglutinating antibody in Ghana, 1970.

RESULTS

1. *Titer distribution.*

Antibody titer distribution is summarized in Table 2 and illustrated in Figure 1, specified by the regions examined. The highest titer obtained was 1: 64. Only 2 persons had this titer, one from the Eastern Region and the other from the Upper Region. As a whole, the antibody level of Ghanaian population was very low. Out of 575 sera examined 395 (68.0%) were negative at the lowest serum dilution, namely at 1: 2. Eighty-five persons (15.0%) were positive at 1: 2. Fifty-four persons (9.0%), 24 (4.0%), 14 (2.4%), 3 (0.5%) and 2 (0.3%) were positive at 1: 4, 1: 8, 1: 16, 1: 32 and 1: 64 respectively. Distribution pattern of the antibody titer in the Greater Accra and the Upper Regions, was almost the same as shown in figure 1. In the Eastern Region, the titer was slightly higher and a small peak was noted at a titer of 1: 4. Geometric mean titer expressed as

TABLE 3
Incidence of cholera agglutinating antibody in Ghana specified by age, titer and region.

Region	Age group (year)	Number tested	Number positive at a titer of					Geometric mean titer (mean well number)*
			1:2≤	1:4≤	1:8≤	1:16≤	1:32≤	
Greater Accra	0-4	20	1(5)	0	0	0	0	0.05
	5-14	34	3(9)	2(6)	1(3)	1(3)	0	0.21
	15-44	132	48(36)	26(20)	11(8)	4(3)	2(2)	0.69
	45≤	44	7(16)	3(7)	3(7)	1(2)	0	0.32
	Total	230	59(26)	31(13)	15(7)	6(3)	2(1)	0.49
Eastern	0-4	2	0	0	0	0	0	0.0
	5-14	12	4(33)	2(17)	2(17)	2(17)	0	0.83
	15-44	69	36(52)	26(38)	9(13)	3(4)	1(1)	1.09
	45≤	25	17(68)	12(48)	8(33)	3(12)	1(4)**	1.68
	Total	108	57(53)	40(37)	19(18)	8(7)	2(2)	1.18
Upper	0-4	15	2(13)	0	0	0	0	0.13
	5-14	61	13(21)	4(7)	1(2)	0	0	0.29
	15-44	122	20(25)	13(11)	3(2)	1(1)	0	0.39
	45≤	39	21(54)	9(23)	5(13)	4(10)	1(3)**	1.05
	Total	237	66(28)	26(11)	9(4)	5(2)	1(0.4)	0.45
Whole Ghana	0-4	37	3(8)	0	0	0	0	0.08
	5-14	107	20(19)	8(7)	4(4)	3(3)	0	0.33
	15-44	323	114(35)	65(20)	23(7)	8(2)	3(1)	0.06
	45≤	108	45(42)	24(22)	16(15)	8(7)	2(2)**	0.90
	Total	575	182(32)	97(17)	43(7)	19(3)	5(1)	0.60

N.B. Figure in brackets indicates percentage.
* Initial dilution of serum was 1:2. ** Final titer was 1:64.

a mean well number was 0.49 for the Greater Accra Region, 1.18 for the Eastern Region and 0.45 for the Upper Region. That for the whole of Ghana was calculated as 0.60 as shown in table 3.

2. *Age distribution of the antibody.*

Age distribution of cholera agglutinating antibody is summarized in Table 3 and Figure 2. In the age group of 0–4 years, the highest titer obtained was 1:2. Only 3 persons (8%) out of 37 tested had this titer. With the advance of age, incidence of antibody production increased and titer varied in wider range. In the age group of 45 years and over, 42% were positive at 1:2 or more and 22, 15, 7 and 2% were positive at 1:4, 1:8, 1:16 and 1:32 or more respectively. Regarding the regions, higher incidence was recorded in the Eastern Region than in the others. Geometric mean titer increased with age. In the age group of 45

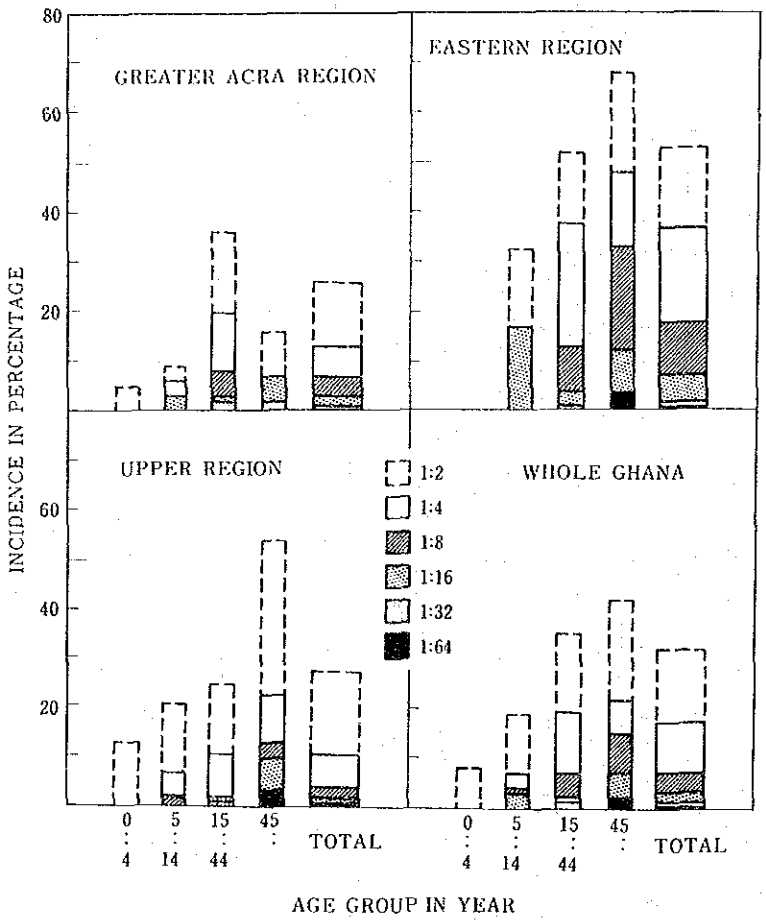

FIG. 2. Age distribution of cholera agglutinating antibody in Ghana, 1970.

years and over in the Eastern Region, the geometric mean titer was calculated as 1.68, which was the highest of all the age groups.

3. *Comparison of antigens.*

Swiss cholera vaccine and a preparation of heat killed vibrio cells were compared for their potency as antigen in agglutination reaction. In the control test using pre- and post-vaccination sera, both antigens gave negative results for the pre-vaccination serum. Titers of the post-vaccination serum were 1:32 for the vaccine antigen and 1:64 for the heat killed vibrio antigen. The general population showed very low level of antibody when the vaccine was used as an antigen. The highest titer obtained was 1:4. Only 3 persons had this titer, 8 persons were positive at 1:2 and the remaining 554 persons gave negative results. On the other hand, using the heat killed vibrio cells as an antigen, the highest titer obtained was 1:64 and titer distributed widely from 1:2 to 1:64 as shown in figure 3. Negative results were obtained in 393 persons. The geometric mean

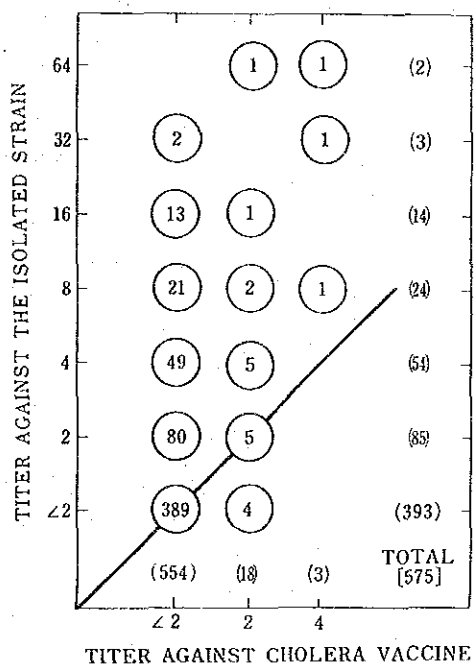

FIG. 3. Comparision of two antigens: an isolated strain of Vibrio cholerae and cholera vaccine.
Figure in cicle indicates number of sera at each titer. Figure in brackets indicates. sum-up number of sera at each titer.

titers expressed as mean well number were 0.60 for the isolated strain and 0.02 for the cholera vaccine.

DISCUSSION

The agglutinating antibody titer of given sera varies according to the antigen used. The titer is expected to become higher when living cells of *V. cholerae* are used than when killed cells were used as an antigen[5]. In a control experiment, the antibody titer of the post-vaccination serum was determined as 1:64 using an antigen of heat killed *V. cholera*, which was isolated from the first case of cholera here in Accra by Dr. Salles. The titer of the same serum was reduced to 1:32 by using cholera vaccine as an antigen. The titers of some sera in the present study might, therefore, be increased if proper living cells were used as an antigen.

In the case of cholera infection, the agglutinin starts to rise in titer on the 4th day after the onset, reaching the highest level in 10–25 days and falling significantly one month after the onset. It could, however, be still detectable 6 months after the onset[4]. From the above considerations, the present results suggest that the general population of Ghana had not been affected greatly by cholera at least 6 months before the collection of specimens.

SUMMARY

Five hundred and seventy-five Ghanaian sera collected before the cholera epidemic in 1970 were investigated for their cholera agglutinin using as an antigen heat killed *V. cholerae* which was isolated from the first case of cholera in Ghana 1970. Antibody level of the general population in Ghana was found to be very low at the time of collection of the sera. Geometric mean titer of these 575 sera was less than 1:2. It was suggested seroepidemiologically that no cholera epidemic had occurred in Ghana at least during the period of 6 months before the collection of serum specimens.

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[I-12] A PRELIMINARY STUDY ON SEROEPIDEMIOLOGY OF VIRAL INFECTIONS IN GHANA*

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It has been strongly suspected clinically and epidemiologically that various kinds of viral diseases would be as common in Ghana as in the other tropical countries, especially polio, smallpox, measles, yellow fever and infectious hepatitis[1]. However, only a few laboratory investigations have been carried out. By examining 340 sera taken from Ghanaians, Pacsa and Amoah[2] confirmed that the Ghanaian had complement fixing antibodies against influenza A, B and C, parainfluenza 1, RS, mumps, herpes simplex, and adenoviruses. No data have been published on neutralizing antibodies of Ghanaians against polio and measles viruses.

In December 1968, sera were collected from 86 Ghanaians in Accra and were brought to us in Japan. Using these sera, neutralizing antibodies against polio 1, 2 and 3, coxsackie B-5, adeno-3 and measles viruses were determined. Hemagglutination inhibition (HI) antibody titers were also determined against influenza, rubella and Japanese encephalitis viruses. Complement fixing (CF) antibodies against adeno and herpes simplex viruses were also studied on these sera.

* This study was partially supported by the Government of Japan under the Medical Cooperation Programs between Ghana and Japan.

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MATERIALS AND METHODS

Serum specimens: In December 1968, Ghanaian sera which were submitted to the Korle Bu Hospital Laboratories for Kahn, Widal and the other blood tests were collected in Accra, brought to Japan in an ice jar and kept at –20°C in our laboratory until use. Four age groups, 1–10, 11–20, 21–30 and 31 and over, were given to arrange them for the tests.

Serological tests: All the tests were carried out by means of microtiter techniques[3]. The antibody titers were expressed as the highest serum dilution at which the positive reaction was finally recognized.

Neutralization (NT) tests for polio, coxsackie B–5 and adenoviruses were performed using HEp–2 cells. Vero cells were employed in measles neutralization test. Tissue culture cells were propagated and maintained in EME medium with 2% calf serum and proper amounts of antibiotics. Amounts of 100 TCD_{50}/0.025 ml of prototype strains of viruses were used as antigens.

Complement fixation (CF) antibodies against adeno–3 and herpes simplex viruses were determined using 4 units of complement and 4 units of antigen after inactivating serum specimens at 56°C for 30 minutes.

Hemagglutination inhibition (HI) test was performed using 4 HA units of antigen. For rubella HI test, the sera to be tested were treated with acid washed caolin to remove non-specific inhibitors. M–33 strain of rubella virus propagated in BHK–21 cells was used as antigen. For influenza HI test, sera were treated with RDE obtained commercially from Takeda Co., Osaka, Japan. The antigens were prepared by infecting cholioallantoic fluid of chicken eggs. Strains used were A2/Murakami/4/64, A2/Kumamoto/1/67, A2/Aichi/2/68 (a Japanese strain of Hongkong type) and B/Tokyo/1/67. For HI test of Japanese encephalitis (JE) virus, non-specific inhibitor of sera was eliminated by acetone treatment. JAGAR strain was used as antigen, which was available commercially as a freezing dried ampule (Takeda Co.).

RESULTS

1. *Polio NT antibodies.*

As summarized in Table 1 and Figure 1, the average incidence of polio NT antibodies at the lowest screening level of 1:4 was estimated to be 92% for type 1, 88% for type 2 and 93% for type 3. By age 10, the positive incidence reached 80% for type 1, and 100% for both types 2 and 3.

In the screenings at higher titers, namely 1:16 and/or 1:64, the incidence tended to decrease gradually as age advanced, to be followed by slight rise in the oldest age group of 31 years and over. Such a tendency was especially marked in types 2 and 3.

TABLE 1
Neutralizing antibodies against polioviruses in 1968.

Age group	Number tested	Type 1			Type 2			Type 3		
		1:4≤	1:16≤	1:64≤	1:4≤	1:16≤	1:64≤	1:4≤	1:16≤	1:64≤
0-10	10	8 (80)*	8 (80)	6 (60)	10 (100)	7 (70)	6 (60)	10 (100)	9 (90)	6 (60)
11-20	27	27 (100)	18 (67)	10 (37)	25 (93)	13 (48)	4 (15)	24 (89)	15 (56)	5 (19)
21-30	29	27 (93)	17 (59)	8 (28)	25 (86)	14 (48)	3 (10)	26 (90)	11 (38)	2 (7)
31≤	20	17 (85)	12 (60)	9 (45)	16 (80)	9 (45)	4 (20)	20 (100)	8 (40)	2 (10)
Total	86	79 (92)	55 (64)	33 (38)	76 (88)	43 (50)	17 (20)	80 (93)	43 (50)	15 (17)

* Figure in brackets indicates percentage.

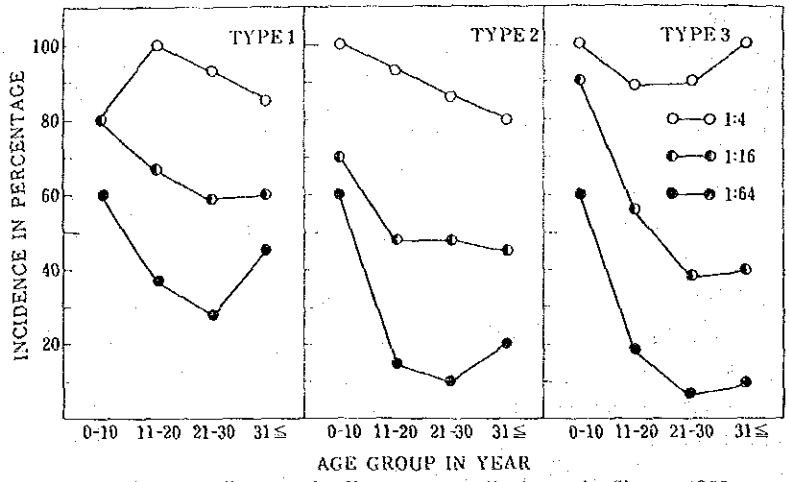

FIG. 1. Neutralizing antibodies against polioviruses in Ghana, 1968.

2. *Coxsackie B-5 NT antibody.*

As shown in Figure 2 and Table 2, rather low incidence was recorded for the NT antibody against coxsackievirus type B-5. At the lowest level of 1:4, 25 persons (31%) out of 80 examined were positive. At a higher level of screening, 2 persons (3%) were positive at 1:16, and only one (1%) was at 1:64. As a whole, the occurrence of positive persons tended to increase with age.

3. *Adeno-3 NT antibody.*

An average incidence of 79% was noted as positive for adeno-3 NT antibody at a level of 1:4. The summarized results are given in Table 2 and Figure 2. By

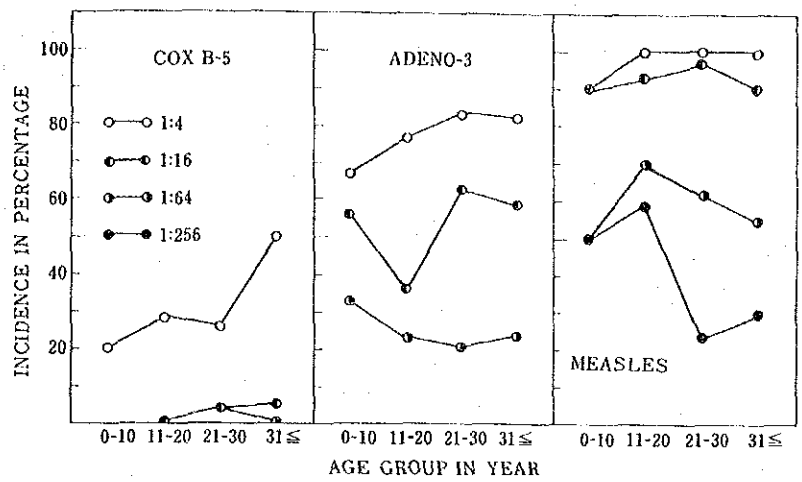

FIG. 2. Neutralizing antibodies against coxsackievirus type B-5 adenovirus type 3 and measlesvirus in Ghana, 1968.

age 10, 67% acquired the antibody, the rate increasing gradually as age advanced.

4. *Measles NT antibody.*

As shown in Table 2 and Figure 2, only 1 person out of 86 exmained was found to have no antibody against meales virus at a level of 1:4, indicating 99% positive. By age 10, almost all of the people acquired the antibody. Extremely high titers such as 1:256 or more were marked in 50% of 0–10 years group and 59% of 11–20 years group.

5. *Adeno CF antibody.*

Adenovirus common CF antibody was titrated on 77 persons using adenovirus type 3 as an antigen. As indicated in Figure 3 and Table 3, the average incidence of positive persons was rather low. At a lower level of screening, 1:4, positive rate was noted as 25%. At a higher level, it was only 9% at 1:8, and 7% at 1:16. By age 10, 20% acquired the antibody at a level of 1:4. The maximum incidence, 36%, was noted in the age group of 21–30 years.

6. *Herpes CF antibody.*

As presented in Table 3 and Figure 3, an average incidence of positive reaction was 39% at a level of 1:4. With increasing ages, the incidence of positive persons was noted to increase.

7. *Influenza HI antibodies.*

As summarized in Figure 4 and Table 4, an average incidence at a level of 1:8 was recorded as high as 82% with A2/Murakami/4/64, representative strain of A2

TABLE 2

Neutralizing antibodies against coxsackievirus B–5, adenovirus type 3 and measles virus in Ghana, 1968.

Age group	Number exam'd	Coxsackie B–5			Number exam'd	Adeno 3			Number exam'd	Measles			
		1:4≤	1:16≤	1:64≤		1:4≤	1:16≤	1:64≤		1:4≤	1:16≤	1:64≤	1:256≤
0-10	10	2 (20)*	0	0	9	6 (67)	5 (56)	3 (33)	10	9 (90)	9 (90)	5 (50)	5 (50)
11-20	25	7 (28)	0	0	22	17 (77)	8 (36)	5 (23)	27	27 (100)	25 (93)	19 (70)	16 (59)
21-30	27	7 (26)	1 (4)	1 (4)	24	20 (83)	15 (63)	5 (21)	29	29 (100)	28 (97)	18 (62)	7 (24)
31≤	18	9 (50)	1 (5)	0	17	14 (82)	10 (59)	4 (24)	20	20 (100)	18 (90)	11 (55)	6 (30)
Total	80	25 (31)	2 (3)	1 (1)	72	57 (79)	38 (53)	17 (24)	86	85 (99)	80 (93)	53 (62)	34 (40)

* Figure in brackets indicates percentage.

TABLE 4

Haemagglutination inhibition (HI) antibodies against influenzaviruses in Ghana, 1968

Age group	Number tested	A2/Murakami/4/64			A2/Kumamoto/1/67			A2/Aichi/2/68			B/Tokyo/1/67		
		1:8≤	1:32≤	1:128≤	1:8≤	1:32≤	1:128≤	1:8≤	1:32≤	1:128≤	1:8≤	1:32≤	1:128≤
0-10	10	9 (90)*	6 (60)	4 (40)	6 (60)	2 (20)	0	3 (30)	1 (10)	0	1 (10)	0	0
11-20	21	20 (96)	19 (91)	14 (67)	14 (67)	3 (14)	3 (14)	10 (18)	2 (10)	1 (5)	3 (14)	2 (10)	1 (5)
21-30	22	17 (77)	12 (55)	7 (32)	6 (27)	3 (14)	0	5 (23)	0	0	1 (5)	0	0
31≤	17	11 (65)	6 (35)	2 (12)	1 (6)	0	0	3 (18)	0	0	2 (12)	1 (6)	0
Total	70	57 (82)	43 (61)	27 (39)	27 (39)	8 (14)	3 (4)	21 (30)	3 (4)	1 (1)	7 (10)	3 (4)	1 (1)

* Figure in brackets indicates percentage.

TABLE 3
Complement fixing (CF) antibodies against adenovirus and herpes simplex virus in Ghana, 1968.

Age group	Number tested	Adeno CF			Herpes CF		
		1:4≤	1:8≤	1:16≤	1:4≤	1:8≤	1:16≤
0-10	10	2 (20)*	0	0	3 (30)	2 (20)	0
11-20	24	5 (20)	1 (4)	1 (4)	6 (25)	2 (8)	0
21-30	25	9 (36)	4 (16)	3 (12)	11 (44)	4 (16)	1 (4)
31≤	18	6 (33)	2 (11)	1 (5)	10 (55)	4 (22)	2 (11)
Total	77	19 (25)	7 (9)	5 (7)	30 (39)	12 (16)	3 (4)

* Figure in brackets indicates percentage.

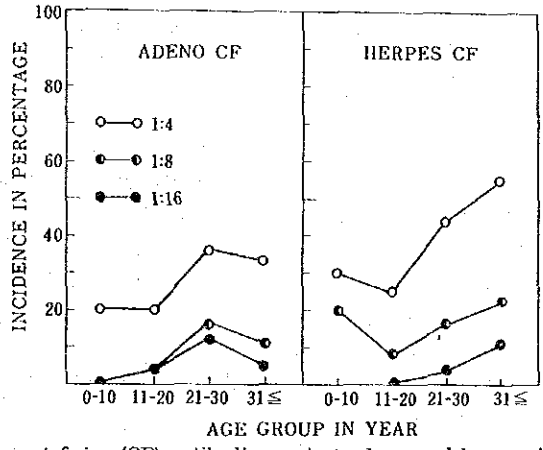

FIG. 3. Complement fixing (CF) antibodies against adeno- and herpes simplex viruses in Ghana, 1968.

influenza virus which had caused wide-spread epidemics in Japan from 1964 to 1967. The incidence was 39% with A2/Kumamoto/1/67, a Japanese strain with antigenic structures and slight modification of Murakami strain, 30% for A2/Aichi/2/68, representative Japanese strain of Hongkong type of influenza and 10% for B/Tokyo/1/67, a current Japanese strain of type B influenza virus.

A remarkable high incidence was noted for Murakami strain in the age group of 11–20 years, at all 3 different levels of screening; namely 96% at 1:8 91% at 1:32 and 67% at 1:128. Antibody against Hongkong type of influenza virus was ascertained to exist among the Ghanaians to some extent at the end of 1968.

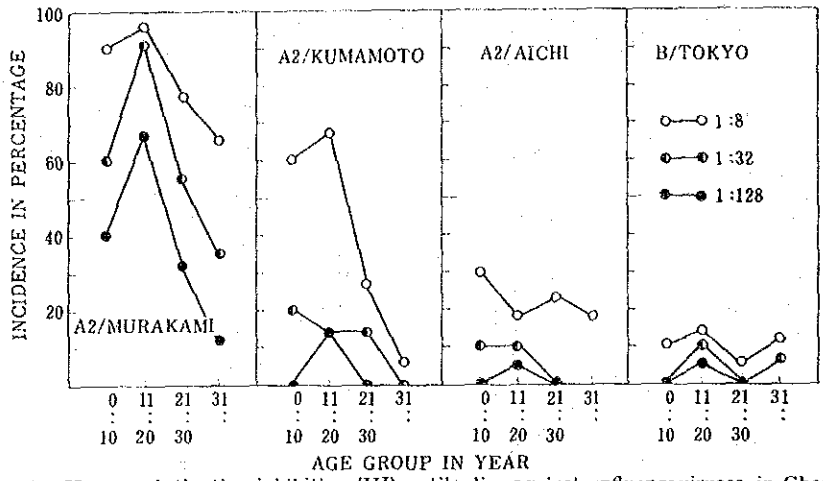

FIG. 4. Haemagglutination inhibition (HI) antibodies against unfluenzaviruses in Ghana, 1968.

The incidence of type B antibody was lower than that of any other type of A2 influenza virus.

8. *Rubella HI antibody.*

An average incidence of 63% was noted for rubella HI antibody as shown in table 5 and figure 5. By age 10, only one person (11%) out of 9 tested acquired the antibody. By age 20, the incidence rose to 71%.

TABLE 5
Haemagglutination inhibition (HI) antibodies against rubella and Japanese encephalitis viruses in Ghana, 1968

Age group	Number tested	Rubella HI			Number tested	Japanese enceph. HI		
		1:8≤	1:32≤	1:128≤		1:10≤	1:40≤	1:160≤
0-10	9	1 (11)*	0	0	6	0	0	0
11-20	21	15 (71)	14 (67)	7 (63)	19	7 (37)	5 (26)	1 (5)
21-30	20	13 (65)	10 (50)	4 (20)	21	6 (29)	6 (29)	5 (24)
31≤	10	8 (80)	5 (50)	2 (20)	16	5 (31)	3 (19)	2 (12)
Total	60	37 (63)	29 (48)	13 (20)	62	18 (29)	14 (23)	8 (13)

* Figure in brackets indicates percentage.

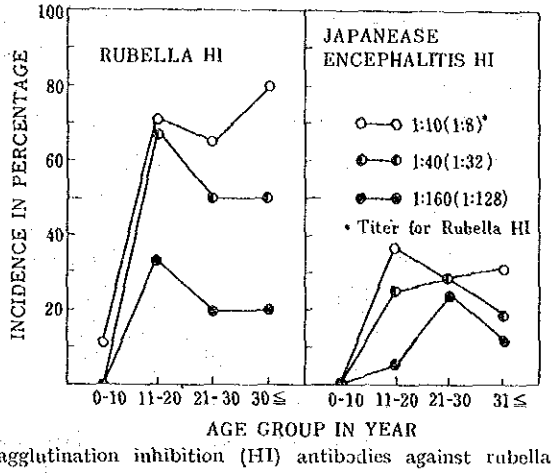

FIG. 5. Haemagglutination inhibition (HI) antibodies against rubella and Japanese encephalitis viruses in Ghana, 1968.

9. *Japanese encephalitis HI antibody.*

As indicated in Table 5 and Figure 5, 18 persons (29%) out of 62 examined were found to have HI antibody against Japanese encephalitis virus. By age 10, none of 6 persons examined was found to have acquired the antibody.

DISCUSSION

It is obvious that the serum specimens obtained for the present study were not sufficient in both number and area covered to give a proper seroepidemiological view on viral diseases in Ghana. Neverthless, the present results may be interesting at least as a fact report in this field of studies.

Poliovirus infection is now accertained to occur widely in Ghana by revealing neutralizing (NT) antibodies extensively among the Ghanaian population. In contrast to the high incidence of polio NT antibodies, a very low incidence of coxsackie B-5 NT antibody was revealed. The high prevalence of polioviruses in the population might interfere with the other enterovirus infection. The authors had no information on the general administration of oral polio vaccines in this country before 1968. The high prevalence of polio-neutralizing antibodies would, therefore, be due to natural infections by wild types of poliovirus.

The high prevalence of measles neutralizing antibody revealed by the present study indicates high invasion of measles virus in the communities of this country. This extremely high invasion might explain to some extent the high mortality of infants and young children in Ghana.

Incidences of adeno common and herpes CF antibodies were 25% and 37%, respectively. These figures accord well to Pacsa's[2].

One of the most interesting findings in the present investigation is the high prevalence of haemagglutination inhibition (HI) antibodies against strains of A2 influenzavirus such as A2/Murakami/4/64, which was one of the predominant influenza-strains in Japan during the period covering 1964–1967. Another interesting finding is concerned with A2/Aichi/2/68, one of representative Japanese strains of Hongkong type of influenza. It is an interesting fact that persons who had HI antibody against this new type of influenza virus were found in Ghana at that time of 1968, though the number of such persons was limited. In Japan, the epidemic of Hongkong type of influenza was reproted early in 1968 in some districts such as Nagoya (Aichi Prefecture) and Tokyo. In our prefecture, Fukushima, however, occurrence of this type of influenza had, not been confirmed by the end of December 1968, when the sera for the present study were collected in Ghana.

Haemagglutination inhibition antibodies against Japanese encephalitis (JE) virus obviously cannot be considered to be specific for JE virus; probably they are products of reaction common to group B arboviruses.

SUMMARY

Examination of 86 sera collected in Accra, Ghana, in December 1968, demonstrated the following viral antibodies

1) Neutralizing antibodies against poliovirus types 1 (92%), 2 (88%) and 3 (93%), coxsackievirus B–5 (31%), adenovirus type 3 (79%) and measlesvirus (99%).

2) Complement fixing antibodies against adenovirus (25%) and herpes simplex virus (39%).

3) Haemagglutination inhibition antibodies against strains of influenzavirus such as A2/Murakami/4/64 (65%), A2/Kumamoto/1/67 (39%) A2/Aichi/2/68, a Japanese strain of Hongkong type of influenza (30%) and B/Tokyo/1/67 (10%), rubellavirus (63%) and Japanese encephalitis virus (29%).

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[I-13] AUSTRALIA ANTIGEN AMONG PATIENTS WITH JAUNDICE IN GHANA

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INTRODUCTION

Australia (Au) antigen discovered by Blumberg *et al.*[1] has been ascertained through various investigations to be an agent closely associated with serum hepatitis rather than infectious hepatitis in classical terminology[2]. The main part of these investigations has been concerned with temperate countries. Only a little information is available from tropical countries, where an extremely high incidence of Au antigen was found[3–5]. Shulman *et al.*[6] reported that Au antigen was detected by complement fixation test at an incidence of 59% among the cases of endemic hepatitis from Ghana, where infectious hepatitis is one of the commonest viral diseases[7]. More than 5,000 cases of this disease were reported in 1969, and the actual number of cases was assumed to be more than 7,000 for 1970[8]. On the other hand, yellow fever cases occur sporadically in Ghana throughout the year. It is rather difficult to distinguish clinically between infectious hepatitis and yellow fever, especially when they occur sporadically and atypically.

From March to December 1970, serum specimens taken from 269 Ghanaians for yellow fever serological tests were sent to the virology laboratory. These sera were examined both for Au antigen and for yellow fever antibodies. The present report is concerned with the results obtained with these sera.

MATERIALS AND METHODS

Serum Specimens.

Two hundred and sixty-nine serum specimens were received for yellow fever

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serological tests from various regions of Ghana from March to December 1970. Some of these sera, especially those from the Upper Region, were sent by mail without refrigeration, which took several days before arriving at the laboratory. The sera were kept at -20°C until the tests were performed. Most of them were taken from the patients within 2 weeks after the onset. Provisional clincial diagnosis described in request cards for virological tests was classified into five categories as shown in Table 1. Jaundice appeared to be a common syndrome to cover all these patients examined.

TABLE 1
Australia antigen and yellow fever antibody in patients with jaundice classified by clinical diagnosis and signs.

Clinical diagnosis and signs	No. of cases examined	Number positive	Incidence in %
Infectious hepatitis	104	35(1)*	33.6(1.0)*
Infectious hepatitis with jaundice	84	31(3)	36.9(3.6)
Jaundice	37	12(1)	32.4(2.7)
Jaundice with fever	22	9(0)	40.9(0)
Yellow fever	22	4(4)	18.2(18.2)
Total	269	91(9)	33.8(3.4)

* Yellow fever cases confirmed by serological tests.

Standard Antigen and Antibody.

Standard Au antigen and its antibody were kindly provided by Dr. B.S. Blumberg, Institute for Cancer Research, Philadelphia (antigen: 66540, antibody: 68571), Dr. A.M. Prince, New York Blood Center, New York (antigen: 70-5623, antibody: No. 1) and Dr. A. Zuckerman, London School of Hygiene and Tropical Medicine, London (antigen: 2716, antibody: 5555). Using these standard materials, working Au antigen and antibody were selected locally. Ghanaian sera (B-5709 and B-032) were selected as working antigen, and Co-0614 and Co-1317 were used as working antibody.

Yellow fever antigen (Y.F.-S.A. -1418/70) and hyperimmune monkey serum against yellow fever virus were kindly supplied by Dr. Y. Robin, Pasteur Institute, Dakar.

Laboratory Methods.

Au antigen was detected by immunoelectrosyneresis technique as described by Prine and Burke[9]. Ouchterlony's agar gel diffusion test[10] was performed only for standardization and also for confirmation of positive cases. The yellow fever hemagglutination inhibition (HI) test was performed by micromethod[11].

RESULTS

1. *Relationship between clinical diagnosis and Au antigen.*

Provisional diagnosis given by clinicians on request cards were classified into five clinical groups as shown in Table 1: infectious hepatitis, infectious hepatitis with jaundice (presumed to be an infectious hepatitis case with remarked syndrome of jaundice), jaundice, jaundice with fever, and yellow fever.

In the clincial group of infectious hepatitis, out of 104 cases 35 (33.6%) were positive for Au antigen and only 1 (1.0%) for yellow fever. In the clinical group of infectious hepatitis with jaundice, 31 (36.9%) out of 84 patients were associated with Au antigen, and 3 cases (3.6%) were ascertained to be yellow fever. In the clinical group of jaundice, 12 (32.4%) patients out of 37 had Au antigen in their sera and only 1 case (2.7%) was proved to be a yellow fever case. In the clinical group of jaundice with fever, 9 (40.9%) out of 22 cases were positive for Au antigen, but no confirmed cases of yellow fever were found. In the clinical group of yellow fever, 4 (18.2%) out of 22 cases examined were positive for Au antigen and also 4 (18.2%) for yellow fever antibody. None of the cases was proved to have both Au antigen and yellow fever antibody at the same time. After all, 91 (33.8%) and 9 (3.4%) out of 269 cases were positive for Au antigen and yellow fever antibody, respectively.

2. *Age distribution of Au antigen in patient with jaundice.*

The nine confirmed cases of yellow fever and patients of uncertain age were excluded. Thus, 236 cases were arranged by age group as shown in Table 2. In patients under the age of 14 years, Au antigen was detected at an incidence of 58.5%. In the age group of 0–4 years, 8 (53.4%) out of 15 were associated with

TABLE 2
Frequency of Australia antigen in patients* with jaundice specified by age group.

Age group	Number examined	Number positive	Frequency in %
0-4	15	8	53.4
5-9	19	12	63.2
10-14	19	11	57.9
Sub-total	53	31	58.5
15-19	36	10	27.8
20-29	81	30	37.0
30 & over	66	13	19.7
Sub-total	183	53	29.0
total	236	84	35.6

* Excluded yellow fever cases and patients of uncertain age.

Au antigen. In the age group of 5–9 years, 12 (63.2%) out of 19 and in the age group of 10–14 years, 11 (57.9%) out of 19 patients were found respectively to be associated with Au antigen. In the older age groups, the incidence of Au antigen was 27.8% for 15–19 years, 37.0% for 20–29 years and 19.7% for 30 years and over. The difference of the incidence between the age groups under 14 years old and over 15 years of age was significant statistically.

DISCUSSION

The present investigation carried out in Ghana revealed that Au antigen was associated with about 1/3 of jaundice cases which were diagnosed clinically as infectious hepatitis or yellow fever. Shulman *et al.*[6] reported that Au antigen was detected in 59% out of 112 cases with a total of 296 specimens taken during the course of diseases of endemic hepatitis in Ghana. They used the complement fixation test which was more sensitive than the method of immunoelectrosyneresis that we employed in the present study. The 2 figures of 34% obtained by us and the other 59% given by Shulman *et al.*, would be comparable with each other if the methods employed were taken into consideration. Infectious hepatitis has been believed to occur very frequently in Ghana, but serum hepatitis in classical terminology has not been given enough attention. However, the present results together with those of Shulman *et al.*[6] suggest that at least more than 34–59% of jaundice cases diagnosed as endemic hepatitis, infectious hepatitis or yellow fever may be associated with Au antigen.

Another important finding in the present study is that Au antigen associated with jaundice cases was found not only in adults but also in children under 14 years of age including infants at an incidence of 58.5%. This fact is very contradictory to what was found in the Western countries, where Au antigen was detected usually in adults over 20 years of age[12].

On the other hand, the prevalence rate of Au antigen in healthy population and non-jaundiced patients in Ghana was approximately 6%, and it rose as age advanced[13]. These facts together with the present results suggest that the mode of transmission of Au antigen in the tropical countries might be different from that in the temperate countries[4]. As already suggested by earlier investigators, vector born transmission might play an important role in spreading the antigen widely among the population in the tropics[2]. No direct evidence has, however, been successfully given to support this attractive hypothesis.

SUMMARY

Both Australia (Au) antigen and yellow fever antibodies were examined on 269 sera sent to the laboratory from various parts of Ghana for yellow fever serology tests. Ninety-one cases (33.8%) were associated with Au antigen and 9 cases (3.4%) were ascertained to be yellow fever. Their clinical diagnosis was

infectious hepatitis, jaundice and/or yellow fever. In children under 14 years of age, including infants, the incidence of Au antigen was 58.5%.

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[I-14]

ENTEROVIRUSES IN INFANTS IN ACCRA—A PRELIMINARY REPORT

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Introduction

A number of studies concerning the infection of enteroviruses in healthy children have been reported in the past two decades (1–6). These reports provided valuable information on the prevalence of enteroviruses in the general population. However, most of these researches were conducted either in temperate or sub-tropical areas; there is very little information about the prevalence of enteroviruses in the tropical zones, particularly in tropical Africa (7–10).

On the other hand, it was pointed out that cases of poliomyelitis have been increasing gradually in many tropical countries in the last ten years (11–13).

In October, 1971, a continuous study of the prevalence of enteroviruses was launched by the Virology Unit of the Department of Microbiology, to obtain basic information about "enterovirus infection" in healthy infants in Accra. A preliminary report on the results so far obtained is presented here.

Materials and Methods

Forty-five infants, aged one to ten months (average age at the beginning of the study, October, 1971, was between 3 to 5 months) were selected from amongst a number of infants attending two Urban Health Centres, Ussher and Kaneshie Polyclinics. The former caters for patients from Ussher and James Town areas, which areas are rather densely populated. The latter Polyclinic which is suburban, caters for patients from the Kaneshie Estate area, an area which has recently been developed and therefore enjoys some modern amenities. It is sparsely populated in contradistinction to the former centre.

All infants in the study-group had each received 3 intra-muscular doses of "Quinto Virelon", a vaccine containing the toxoids of C. diptheriae and Cl. tetani, inactivated bacillus of B. pertussis, inactivated poliomyelitis and measles viruses, at 4-weekly intervals. The vaccine was kindly supplied by the Behringwerke AG. Co. through its representative in Ghana, Hoechst, Ag. Accra. From October, 1971 to August, 1972, faecal specimens were collected from infants in the study group once every two months. Stool containers were distributed to each household and the specimens were collected the following morning. The specimens were kept at —20°C until treated for inoculation.

In the laboratory, 10 to 20% of faecal suspensions were prepared in cold transportation medium (Earle's BSS containing 0.5% lactalbumin hydrolysate, supplemented with bovine plasma albumin (0.1%) and fortified with antibiotics). The faecal suspensions were centrifuged in the cold at 2,000 r.p.m. for 15 to 20 mins. to obtain a clear supernatant. HEp-2, HeLa and Vero cells cultured in tubes were each inoculated in duplicate with 0.1ml. per tube of faecal extract. The inoculated cells were incubated at 37°C and were observed daily for CPE for a period of 7 to 10 days. Cultures were harvested and passaged at least twice if no CPE was observed. Cultures showing CPE were harvested when the CPE had reached a maximum; they were stored at —20°C until the next passage.

The isolates were titrated for their infectivity, then identified serologically using WHO intersecting serum pool scheme (14). An appropriate volume of each serum pool was mixed with an equal volume of diluted test virus containing 300–500 TCID50/0.1ml. The mixtures were incubated at 37°C for 2 hours and 0.2 ml. thereof were inoculated into tube cultures. Observation was made daily for 3—5 days and final reading was usually made a day after the virus controls showed complete CPE.

Results

Virus isolation rates by month:

In Table I, the overall numbers and rates of enterovirus isolation per month, i.e. from October, 1971, to August, 1972, are shown.

TABLE 1: *Virus isolation rates by month*

Sampling	Month	Number of Samples	Number of Isolates	Percentage of Isolation
1	Oct. 1971	45	12	26.7%
2	Dec. 1971	43	26	60.5
3	Feb. 1972	44	12	27.3
4	Apr. 1972	44	27	61.4
5	Jun. 1972	42	12	28.6
6	Aug. 1972	34	16	47.1
Total		252	105	41.7 (s*=16.3)

Note s = Standard deviation*

The total number of isolates was 105 out of a total of 252 specimens examined, so that the average rate of isolation is about 42%. The highest isolation rate, 61.36%, was scored in April, 1972, and the lowest, 26.67%, in October, 1971. It appears from these preliminary results therefore, that there could be no relationship between a particular season and the rate of virus excretion. This statement, however, does not preclude the possibility that with a much bigger sample size a correlation between the seasons and virus excretion rates could be established.

The frequencies of virus isolation from individual infants ranged between 0 and 5; 2 or 3 positive virus isolations were made from the majority of the infants after 6 samplings.

Virus isolation according to Area and month of sampling:

Table 2, which represents the rate of virus isolation according to area and month of sample collection, shows that there was no significant difference between the total virus isolation rate for Ussher Town (densely populated) and Kaneshie Estate (sparsely populated). The highest virus isolation rates recorded for Ussher Town were in December, 1971 and August, 1972; the figures were 68.4% and 73.3% respectively. For the Kaneshie Estate the highest virus isolation rate, 66.6% was recorded in April, 1972. The lowest virus isolation rate was recorded in October, 1971 in Ussher Town. The results reveal that the high virus isolation rate for Ussher Town (68.4%) and for the Kaneshi Estates (54.2%) in December, 1972 was due to a "Silent Spread" of ECHO-19 probably throughout the city. From these results, it could be inferred that during an epidemic of Enterovirus Infection, children in densely

TABLE 3: *Virus Isolation versus Water and Kitchen Facilities*

Facilities	Condition	No. of Infants	No. of Isolates	Frequency of Virus Isolation per Infants
Water supply	Private	12	24	2.00
	Public	27	74	2.74
Kitchen	Modern K.	23	59	2.56
	Traditional K.	16	39	2.43
Total		39	98	2.51

TABLE 4: *Virus Isolation versus Sanitary Facilities*

Facility	No. of Infants	No. of Isolates	Frequency of Virus Isolation per Infant
Flush Toilet (private) ...	9	12	1.33
Non Flush Toilet (private) ...	9	28	3.11
None ...	21	58	2.76
Total ...	39	98	2.51

TABLE 2: *Virus isolation rate by area and month.*

Month	Ussher Town			Kaneshie Estate		
	No. of Samples	No. of Isolates	Percentage Isolation	No. of Samples	No. of Isolates	Percentage Isolation
Oct. 1971	20	4	20.0	25	8	32.0
Dec. 1971	19	13	68.4	24	13	54.2
Feb. 1972	20	6	30.0	24	6	25.0
April 1972	20	11	55.0	24	16	66.6
June 1972	19	6	31.6	23	6	26.1
Aug. 1972	15	11	73.3	19	5	26.3
Total	113	51	45.1 (s*=22.26)	139	54	38.8 (s*=17.42)

Note s = Standard deviation*

populated areas (e.g. Ussher Town) have a higher risk of infection than children in sparsely populated areas (e.g. Kaneshie Estate).

Virus isolation versus water supply and Kitchen facilities:

Communal water was available to all areas investigated in this study. Out of 19 households in Ussher Town examined, only one household had its own private water tap as against half of the households in the Kaneshie Estate. As shown in Table 3, the frequency of virus isolation amongst infants from households with private water taps was lower than amongst those without, and therefore, depended on public water supplies.

We could however, not establish any significant relationship between enterovirus isolation rates and the "modern kitchen/ traditional kitchen".

Virus isolation rates versus toilet facilities:

Similar to private household water taps, private tiolet facilities were woefully inadequate in Ussher Town. As can be seen from Table 4, the frequency of virus isolation amongst infants from households with flush toilets was markedly lower than amongst infants from households having other forms of toilet facilities.

Enterovirus groups and serotypes:

The serological groups and serotypes of isolates so far tested are set out below:

Serological groups: Eight polioviruses, 2 Coxsackieviruses and 49 Echoviruses were identified. Nine isolates could not be identified using WHO–Schmidt Intersecting Serum Pools, and 36 isolates remain to be typed.

Serological Types: In the poliovirus group, 3 type 1 polioviruses, 2 type 2 polioviruses and 3 type 3 polioviruses were identified. It must be noted, however, that all the 3 polio-3 viruses were isolated in October, 1971; 2 of the 3 polio-1 viruses were isolated in August, 1972.

In the Coxsackievirus group, one each of Coxsackie A-16 and Coxsackie B-6 were isolated in February and in June, 1972 respectively.

The Echovirus group: In December, 1971 alone, 12 strains of Echovirus type 19 (Echo–19) were isolated. No Echovirus type 19 was isolated prior to December, 1971 and none thereafter, except in February when one such isolation was made. On the grounds of these findings, it is in place to postulate that there was an outbreak of a "silent Echo–19 epidemic" among infants in Accra. Echo–7 and Echo–11 were frequently isolated during the course of the present study. These Echoviruses together with Echo–19 accounted for 42% of all Echoviruses identified.

Discussion

The overall enterovirus isolation rates obtained in this study are similar to those obtained in healthy children in Ibadan, Nigeria (8) and from nursery children in Trinidad (9). It is, however, higher than those obtained from infants in Bangui, Central Africa (15). There was on the contrary, a remarkable difference between our results and those obtained from healthy children in the U.S.A. (6).

With the results so far obtained, there seems to be no seasonal influence on the distribution of enteroviruses in the study areas. However, a survey covering only a one year period would not permit, taking various factors into consideration, any firm conclusion to be made as to whether or not the various seasons have any influence on the prevalence of enteroviruses in infants. To answer this question unequivocally, a longer survey period is imperatively called for.

In December, 1971, when many Echo–19 and a few Echo–7 virus strains were isolated from faecal materials collected from infants in Accra, we could at the same time isolate Echo–19 as well as Echo–7 from sewage samples collected from various areas in the Greater Accra Region (Addy and Otatsume, unpublished data). These findings suggest that there was much dissemination of these viruses in the Greater Accra Region at that time.

Poliovirus type 1 isolations in this study were made in June and in August, 1972. Between May and September of the same year, about 50 requests for virological and serological confirmation of poliomyelitis reached our laboratory from the Children's Block, Korle Bu Teaching Hospital, Accra, 8 of which turned out to be poliovirus type 1.

Both study areas from where specimens were collected lacked various social and hygienic facilities, though not equally. The population status of the two study areas is however, different. In Ussher Town, some areas have a population density of more than 600 persons per acre (16). In the Kaneshie Estate area, the population density is

comparatively low. Kaneshie Estate area has better social and hygienic facilities. Yet the difference in the overall virus isolation rates between Ussher Town and the Kaneshie Estate was not significant as indicated in Table 2.

In Table 4, the importance of personal and social hygiene in the spread of enteroviruses in the two study areas is clearly demonstrated. The ratio between the frequencies of virus isolation per infant, coming from a home with no toilet facilities at all, and those with flush toilets was approximately 2: 1. On the other hand, the ratio between the frequencies of virus isolation per infant, coming from a home with toilet facilities but of the non-flush type and those with flush toilets was nearly 3: 1. From these figures, it can safely be postulated, keeping the housefly situation in the country in mind, that it is easier for flies to gain access to open non-flush toilets and from there onto exposed foods. This most probably accounts for the high frequency of virus isolation noted for infants coming from homes with private but non-flush toilets. On the other hand, infants from homes with no toilet facilities at all would have to depend on public toilets or have to resort to unhygienic practices by defaecating anywhere in the house. This practice is particularly rampant among children under the age of 5 years. Here too, houseflies would certainly contaminate uncovered foods with enterovirus-infested faecal material and hence the high frequency of enterovirus isolation for this group.

Pacsa and Afoakwa (17), stated that the natural state of enterovirus flora of the general population had not changed in this country. This is because no large scale vaccination against poliomyelitis has ever been carried out in Ghana. Furthermore, it is quite important to obtain a basic information on the prevalence of enteroviruses in the country in order to plan an effective poliomyelitis vaccination campaign and this survey was conceived for the purpose.

Summary

Since October, 1971 a longitudinal study of enterovirus infection of healthy infants of Accra has been going on for one year. The Principal findings so far obtained are as follows:–

1. Overall enterovirus isolation rate was 41.67% (Standard deviation = 16.30%), and seasonal distribution of enterovirus infection was not observed during the study.
2. There was no significant difference of overall virus isolation rate of densely populated area (Ussher town) and sparsely populated area (Kaneshie estate). But when there was an epidemic of certain enterovirus, the more populated area had a higher infection rate.
3. It was observed that improvement of hygienic conditions, such as water services and toilet facilities would decrease the frequency of virus infection in infants.
4. All 3 types of polioviruses and many echovirus types were isolated. The geographical and seasonal relationship of these isolates were discussed.

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[I-15] ガーナのレプトスピラ症

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昭和46年7月から48年4月にかけて，海外技術協力事業団から派遣されて西アフリカのガーナ国における医療協力に従事した。ガーナはもとの英領黄金海岸であり，地勢は北部草原地帯，中西部の熱帯森林地帯そして大西洋ギニア湾に面した海岸草原地帯の三つに大別できる。海岸にある首都アクラでの平均気温は，乾期(12月～2月)で30°C前後，雨期（7～8月)で25°C 程度である。

ところでアクラは，黄熱の病原体はレプトスピラであると主張していた野口英世博士がみずから黄熱に感染して死亡（1928年5月）したその町である。レプトスピラ症は特に熱帯地方においては普通にみられる感染症の1つであり，その重症型を黄熱や流行性肝炎と鑑別することは実際的に重要な問題である。それにもかかわらず，ガーナのみならずアフリカからのレプトスピラ症についての知見は少なく，中央アフリカのザイール（もとベルギー領コンゴ）における van Riel ら[4]の報告(1953～56) が目立つ程度である。ガーナにおいては血清疫学調査が1964年に Rothstein[3]よって行なわれているが，それ以外の報告はみられないように思われる。そこで急性熱性黄疸の患者血清中のレプトスピラ症陽性率についての調査をはじめ，農村の一般住民中のレ症陽性率調査やアクラ市内のイエネズミからのレプトスピラ分離等を試みたので，それらの成績を以下に報告する[1]。

実験材料と方法

1) 急性熱性黄疸患者血清

試験血清は臨床的に流行性肝炎あるいは黄疸と診断された患者の急性期および回復期のペア血清という条件で定期的に収集した。協力してくれたのは中西部森林地帯の Agogo 病院（首都アクラから約270 km）と，海岸草原地帯で東部国境を流れる西アフリカ有数の大河であるボルタ河に近い Adidome 病院（アクラから約150 km）の二つであり，ほかにアクラ市内の Korle Bu 病院（国立中央病院でガーナ大学医学部の教育病院を兼ねる）やアクラ郊外の Legon 病院からの少数の依頼血清が含まれる。

2) 農村住民血清

アメリカのカリフォルニア大学からガーナに派遣されていた Dr. D. Belcher らがガーナ農民の総合的健康調査を計画し，昭和48年1月から3月にかけてアクラ市北方約25 km の農村である Kwashikuma と Odontia でそれを実施していた。その調査で得られた住民の血清について多種目の検査の一つとしてレプトスピラ症血清反応を依頼されたため，帰国直前まで試験を続けた。

3) イエネズミからの「レ」分離

ガーナ医大動物舎，アクラ市海岸のガーナ人住宅，アクラ市中央マーケット等で捕えたイエネズミ（すべてクマネズミ）の腎皮質からレプトスピラ分離培養を試みた。

4) 血清反応

コルトフ培地に2～3週培養したレプトスピラを生抗原として用いる顕微鏡的凝集溶菌反応を実施した。抗原レプトスピラは WHO 専門委員会のすすめる15血清群をそれぞれ代表する血清型を用いた。このうち6株はおのおのの血清型の標準株であり，表1で★印で示されている[5,6]。

血清反応は試験血清と時間を節約するためにまず100倍希釈で試験し，これに対して陽性の反応をみとめた場合に10～3,000倍希釈で再試験をする方法をとった。

Table 1　Leptospiral Strains Used as Antigens

1. RGA* (*Icterohaemorrhagiae* group)
2. Veldrat Batavia 46* (*Javanica* group)
3. Hond Utrecht IV* (*Canicola* group)
4. Ballum (*Ballum* group)
5. Salinen* (*Pyrogenes* group)
6. Cynopteri (*Cynopteri* group)
7. Akiyami A* (*Autumnalis* group)
8. Ballico (*Australis* group)
9. Pomona* (*Pomona* group)
10. Moskva V* (*Grippotyphosa* group)
11. P 40-3705* (*Wolffi* group)
12. Akiyami B (*Hebdomadis* group)
13. Van Tienen* (*Bataviae* group)
14. Hyos (*Tarassovi* group)
15. RS 173 (*Semaranga* group)

*Reference strains recommended by WHO Expert Committee.

成　績

1)　急性熱性黄疸患者血清

流行性肝炎または黄疸と臨床診断された患者で急性期と回復期の２回採血できたのは計38例であった。そのうち11例に血清希釈倍数300倍以上の終末価を認め，レプトスピラ症に対する特異的反応であると考えられた。これら11例中７例は急性期には陰性でその後の抗体価上昇が著るしく，また３例は回復期血清の抗体価が急性期よりも高く，ともに今回の症状がレプトスピラ症によるものと診断された。残りの１例は回復期の方が低い抗体価であったが，終末価が300倍のため陽性と判定した。これらのペア血清における陽性率は，28.9%であった。

症状が軽快するともはや通院しなくなる患者が多く，回復期の採血ができずに急性期血清だけという症例が61例あった。このうち10例が急性期ですでに300倍以上の抗体価を示していたが，陽性率はペア血清の場合のおよそ1/2の16.4%であった（Table 2, 3）。

陽性例の多くは二種以上の血清群のレプトスピラに対して反応したが，そのような場合にも常に他よりも高い抗体価を示すどれか一つの血清群が認められた。計21陽性例の病原レプトスピラを血

Table 2　Hospital Sources of Suspected Cases of Leptospirosis and Related Serological Results.

Name of Hospital	Paired Sera		Single Serum	
	Number of Cases	Number Positive	Number of Cases	Number Positive
Agogo	29	9	31	5
Adidome	8	2	21	3
Korle Bu	1	0	4	1
Legon	—	—	5	1
Total (Incidence Rate)	38	11 (28.9%)	61	10 (16.4%)

Table 3　Leptospiral Serogroups and Antibody-Titre of Positive Cases.

Case No.	Hospital	First Specimen (Acute))	Second Specimen (Convalescent)
1	Agogo	Negative	*Autumnalis*×300 *Bataviae*×100
2	Agogo	*Cynopteri*×100 *Pomona*×100	*Cynopteri*×300 *Pomona*×100
3	Agogo	Negative	*Ballum*×300
4	Agogo	*Bataviae*×1000 *Australis*×100 *Ictero.*×100	*Bataviae*×1000 *Canicola*×300 *Australis*×100 *Ictero.*×100 *Grippo.*×100
5	Agogo	Negative	*Bataviae*×300 *Ictero.*×100 *Tarassovi*×100
6	Agogo	*Tarassovi*×300	*Tarassovi*×1000
7	Agogo	Negative	*Canicola*×300 *Ictero.*×100
8	Agogo	*Tarassovi*×300	*Tarassovi*×1000
9	Agogo	Negative	*Ictero.*×300
10	Adidome	Negative	*Ictero.*×300
11	Adidome	Negative	*Ictero.*×300
12	Agogo	*Canicola*×300	—
13	Agogo	*Tarassovi*×300	—
14	Agogo	*Autumalis*×300 *Ictero.*×100 *Australis*×100 *Pomona*×100	—
15	Agogo	*Bataviae*×1000	—
16	Agogo	*Canicola*×300 *Australis*×100 *Bataviae*×100 *Grippo.*×100	—
17	Adidome	*Ictero.*×300	—
18	Adidome	*Ictero.*×300	—
19	Adidome	*Ictero.*×1000	—
20	Korle Bu	*Ictero.*×3000	—
21	Legon	*Tarassovi*×1000 *Grippo.*×300 *Ictero.*×100 *Bataviae*×100	—

清群別にみると，*Icterohaemorrhagiae* 7例，*Tarassovi* 4例，*Canicola* と *Bataviae* おのおの3例，*Autumnalis* 2例そして *Cynopteri* と *Ballum* おのおの1例であった。

2) 農村住民血清

血清サンプルの性，年齢の確認はできなかったが，調査を実施していたアメリカ人チームの説明によれば，健康で日常生活を営んでいる成人の男女農村住民から採血したということであった。Table 4に示したように，試験した80例中6例に300倍の抗体価を認め，その陽性率は7.5%である。血清群は，*Hebdomadis* 3例，*Icterohaemorrhagiae* 2例，*Wolffi* 1例であった。

Table 4 Antibody to Leptospira among Healthy People in Rural Area.

Number of Cases	Number Positive	Incidence Rate	Leptospiral Serogroups
80	6	7.5%	*Hebdomadis* (3 Cases) *Ictero.* (2 Cases) *Wolffi* (1 Case)

Note: The antibody-titre of all the positive cases was 1 in 300.

3) イエネズミからの「レ」分離

試験した計12匹のイエネズミはすべて家屋内でネズミカゴを用いて生け捕りにしたものである。ガーナ大学動物学教室で同定したところ西アフリカの大型イエネズミはクマネズミ (*Rattus rattus*) だけであり，温帯地方で優勢なドブネズミ (*Rattus norvegicus*) は全くみられないということであった。12匹の捕獲場所は，ガーナ医大動物舎8匹，アクラ市海岸ガーナ人住宅3匹，中央マーケット商店1匹である。いずれも腎皮質小片を小乳鉢内で生理的食塩水と共に押しつぶしてその一滴を暗視野鏡検し，また切り刻んだ腎小片をコルトフ培地3本に接種した。全例が鏡検，培養ともに陰性であった。しかし鏡検では，活発に運動する多数の原虫様微生物を認めたのが6例もあり，特に医大動物舎のネズミでは8匹中5匹が陽性であった。成書で調べ，またガーナ人研究者の意見もきいたところ，これらは *Trypanosoma lewisi* と推定された。

考　察

熱帯アフリカには多種類のレプトスピラ血清型が存在すると思われる。van Riel らのザイール(旧ベルギー領コンゴ) における報告[4]では犬や牛から7種の血清群に属するレプトスピラが発見され，そのうち5種はヒト患者からも発見されたという。ガーナでのこれまでの報告 (Rothsten, 1964)[3]によれば，流行性肝炎の診断で入院した患者117例中65例がレプトスピラ抗原に対して陽性の反応を示している。しかし用いられた抗原がフォルマリン固定のレプトスピラで非特異的凝集反応が時にみられ，また3～4種の血清型を混合したものであり，陽性反応がその中のどの血清型に対しているものであるかを確認する試験は行なわれていない。

ところで WHO Report (1967)[5]によれば既知のレプトスピラ血清型は120種を越えている。従ってこれらの血清型のすべてを血清疫学的調査の抗原として用いることは不可能にちかい。そこで，互いに高い抗体価まで類属反応を示すいくつかの血清型で血清群を構成し，そのおのおのの群を代表する血清型でもって血清反応を行なうことが実際的であると考えられた。著者らは WHO 専門委員会が推選する血清群について試験を実施した。各群の代表的な血清型おのおの一つづつ計15株を用いたが，そのうち9株は WHO のいう標準株である。このような理由で，著者らが今回の調査で確認できたのは陽性例の血清群までであり，個々の血清型ではない。血清型を知るためには，患者あるいは病原体保有動物からレプトスピラを分離同定することが必要になる。アクラ市内のクマネズミで試みた結果はすべて陰性に終った。レプトスピラを証明するためには以下に述べるような理由からも，熱帯雨林地帯での調査が重要と考えられ，このことが次に残された課題である。すなわち Table 3の成績でみると，森林地帯の Agogo 病院からの14陽性例中に7種の血清群が発見されているのに対して，草原地帯に位置する他の二つの病院からの7陽性例中6例までが *Icterohaemorrhagiae* 群であった。森林地帯では宿主になる野生動物の種類と個体数の多いこと

が，病原レプトスピラの種類を多彩にする一つの原因であると思われる。

なお同じ血清群に属する陽性例でも，反応の発現状態に相違がみとめられるものがいくつかあった。例えば *Tarassovi* 群の4例のうち，症例6・8および13は *Tarassovi* 群に対してだけ反応しているのに対して，症例21は *Tarassovi* 群のほか3血清群に対して類属凝集反応を示している。同じような結果は，*Bataviae* 群や *Canicola* 群でもみられた。これは抗原構造の異なる複数のレプトスピラ血清型に重感染したことによるものなのか，あるいは未知の血清型感染の可能性もないとはいえない。

急性期および回復期の対血清の成績をみると，11陽性例中10例に回復期における抗体価の上昇が認められる。この事実で今回の症状がレプトスピラ症によるものであることが証明されよう。しかし急性期だけの血清の場合には，必ずしも今回の感染とは断定できず，既往の感染による抗体の残存という例も考慮する必要がある。また対血清の11陽性例中7例までが急性期において陰性であったのは，レプトスピラ症の場合に発病後一週頃から血中抗体が出現するということで説明できる。急性期だけの単独血清の陽性率が低いことも同じ理由によるものと思われる。すなわち，急性期に陰性であったものの中には，もし回復期に再び試験すれば陽性になる例が含まれると思われる。

次に患者ではなく日常生活をしている農村住民の血清試験では，陽性率7.5%であった。これは日本のレプトスピラ症多発地域における陽性率(7.1%)と同じ程度である[2]。ただ血清群は6陽性例中3例が *Hebdomadis* 群であり，ある特定の血清型レプトスピラが局在的に認められることを暗示している。

まとめ

西アフリカのガーナ国において，99例の流行性肝炎あるいは黄疸と診断された患者血清を，WHO 専門委員会の推選する15血清群をそれぞれ代表する血清型のレプトスピラ生抗原を用いて，顕微鏡的凝集溶菌反応によって試験した。急性期および回復期の対血清38例中11例 (28.9%)，急性期だけの単血清61例中10例 (16.4%) が陽性であった。また健康な農村住民血清80例中6例が陽性で感染率は7.5%であった。

陽性例におけるレプトスピラ血清群は9群すなわち *Icterohaemorrhagiae, Tarassovi, Canicola, Bataviae, Autumnalis, Cynopteri, Ballum*, そして *Wolffi* である。

レプトスピラの分離同定にはいたらず，このため血清型の確認はできなかった。

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LEPTOSPIROSIS IN GHANA

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Leptospirosis is regarded as one of the common infectious diseases in tropical Africa, and it is important to differentiate the severe form of this disease, from other diseases such as infectious hepatitis or yellow fever. However, there are few reports on leptospirosis in West Africa.

We examined in Ghana a total 99 cases, mainly diagnosed as infectious hepatitis or jaundice, providing 38 paired sera and 61 single sera, and then 80 cases of healthy adults in rural area. The microscopic agglutination test technique was used. We used live leptospires as antigens, they were cultivated on Korthof's medium for two or three weeks. Fifteen serogroups of leptospires recommended by the WHO Expert Committee (1967) on leptospirosis were tested against each serum.

Of the 38 cases of paired patient sera, 11 (28.9%) gave positive reactions, and of the 61 cases of single patient sera 10 (16.4%) were positive. Of the 80 cases of healthy people in rural area, 6 (7.5%) were found as positive cases.

Leptospiral serogroups of the positive cases are as follows: *Icterohaemorrhagiae, Tarassovi, Canicola, Bataviae, Cynopteri, Autumnalis, Ballum, Hebdomadis* and *Wolffi*.

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[I-16] CATION CONTENT AND TRANSPORT CHARACTERISTICS OF THE SICKLE-CELL ERYTHROCYTE AND THEIR RELATIONSHIP WITH STRUCTURAL CHANGES IN THE MEMBRANE

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SUMMARY

1. The intra-erythrocytic concentrations of sodium and potassium and the water content have been determined for haemoglobin (Hb) SS cells and negroid Hb AA cells.

2. The erythrocyte concentration of sodium was 40% higher and potassium 10% lower in the Hb SS than in the Hb AA cells. The cell water expressed as % weight of cell (corrected for trapped plasma) was identical for both cell types.

3. Normal Caucasian erythrocytes with Hb AA contained 40–50% less sodium but about the same potassium concentration as negroid Hb AA cells.

4. Potassium efflux into buffered iso-osmotic sucrose medium was much faster in Hb SS than in negroid Hb AA cells; ouabain-sensitive active sodium transport was twice as fast in the sickle-cell erythrocytes. Passive sodium efflux of erythrocytes suspended in a physiological medium was similarly faster in Hb SS cells.

5. Under the conditions of the experiments not less than 85% of the Hb SS erythrocytes appeared biconcave. Electron-microscopic examination of ultra-thin sections of Hb SS cells revealed marked discontinuities in the membrane. This suggests definite membrane alterations, which have probably resulted from the sickling–unsickling cycles occurring during the life-span of the cells.

6. It is suggested that the enhanced active sodium transport in the Hb SS erythrocyte is secondary to the augmented passive cation efflux, which in turn results from the leakiness of the erythrocyte membrane produced by the sickling–unsickling process.

Key words: sickle-cell erythrocytes, sodium, potassium permeability, membrane structure.

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The electrolyte and water composition of human erythrocytes have been reported by many workers (Valberg, Holt, Paulson & Szivek, 1965; Beilin, Knight, Munro-Faure & Anderson, 1966; Czaczkes, Ullmann, Ullmann & Bar-Kochba, 1963; Smith, 1972). These reports have been of investigations performed on Caucasian erythrocytes, and despite the variation in experimental procedures the results have been relatively consistent. There is, however, only scanty information in the literature on the electrolyte content of negroid erythrocytes (Balfe, Cole, Smith, Graham & Welt, 1968).

Several observations have shown that the erythrocyte membrane is a highly effective diffusion barrier for cations, and suggest that the diffusion and active pumping of cations are two parallel and independent processes (Tosteson & Hoffman, 1960; Post, Albright & Dagani, 1967). It has also been shown that anions penetrate the membrane much faster than cations (Wilbrandt, 1940). The discriminatory power of the erythrocyte membrane for selective permeability of ions can therefore be ascribed to the structural and functional integrity of the membrane.

Under certain pathological conditions such as hyperthyroidism (Smith & Samuel, 1970), hepatic cirrhosis, chronic renal disease and congestive cardiac failure (Czackes, Aviram, Keynan & Ullmann, 1967) the erythrocyte shows alterations in trans-membrane exchanges of sodium and potassium. However, few reports have appeared on such exchanges in the sickle-cell erythrocyte (Tosteson, Carlsen & Dunham, 1955; Van Eps, Schouten, Sloof & Van Delden, 1971). Recent investigations of the sickle-cell erythrocyte, using electron microscopy, have shown abnormalities suggesting localized membrane reorganization (Lessin, Jensen & Klug, 1972). The experiments reported here were designed to compare the cation concentrations and the transport characteristics of normal (haemoglobin AA) and sickle-cell (haemoglobin SS) erythrocytes. Electron-microscopic examination of the membranes has also been carried out in an attempt to link the sodium and potassium permeability differences in the two cell types with possible structural differences in the erythrocyte membrane. A preliminary communication of this work has already been reported in abstract (Kurantsin-Mills, Kudo & Addae, 1973).

MATERIALS AND METHODS

Experimental subjects

The normal control Ghanaian subjects (haemoglobin AA) used in these experiments were medical and laboratory personnel and medical students. All the Caucasian subjects had been living under the cool (20°C) and hot (40°C), humid weather conditions of Southern Ghana for not less than 24 months. None of the control subjects (Negro or Caucasian) had any history of any important organic disorder. Their haematological profiles were normal. The sickle-cell anaemic subjects (haemoglobin SS) were selected from patients attending the Sickle-Cell Clinic at the Korle Bu Teaching Hospital, Accra. All the patients were negroid Ghanaians; they were clinically in a steady state with no complaints or evidence of infection. Their packed cell volumes (PCV) ranged between 25 and 40%. There were eighty-six subjects, whose ages ranged from 15 to 45. Of these 40% were females. Blood (10 ml) was withdrawn through a wide-bore needle (with minimal venous compression) into plastic heparinized (heparin, BDH Ltd) syringes. The blood was immediately transferred into cellulose nitrate centrifuge tubes and used for the experiments within 30 min.

Determination of sodium, potassium and water content of erythrocytes

The blood was centrifuged at 1000 *g* for 10 min at room temperature (25°C). Plasma, buffy coat and the very topmost layer of erythrocytes were removed. The remaining erythrocytes were washed three times in 4 vol. of 285·0 mosmol/l $MgCl_2$ buffered with 20·0 mmol/l Tris–HCl buffer to pH 7·4 (wash solution). The cell suspension was centrifuged at 1000 *g* for 10 min and the supernatant aspirated. After the last wash the erythrocytes were suspended in sufficient solution to give a PCV of about 50% and the exact PCV was determined in triplicate thereafter. The cells were haemolysed by diluting the suspension 1:200 with deionized, glass-distilled water, for the determination of sodium and potassium by the use of EEL model 150 flame photometer. Calibration of the photometer was checked frequently with accurately known standards.

The sodium and potassium concentrations of erythrocytes measured by this method have been shown by this study and also by Smith (1972) to be closely comparable with those determined by more complicated methods (Beilin *et al.*, 1966; Czaczkes *et al.*, 1967).

Trapped plasma was determined by the Evans Blue dye (T1824) technique (Hlad & Holmes, 1953) on the blood of eleven control negroid subjects and eleven sickle-cell patients. Percentage of trapped plasma was calculated on thoroughly packed cells.

Erythrocyte water was determined on a sample of thoroughly packed cells dried at 105°C to a constant weight. The water content calculated from the dried cells was corrected for intercellular fluid.

Permeability of Hb AA and Hb SS erythrocytes in sucrose medium

Fresh blood was taken from subjects as described above and washed three times in the wash solution. After the last wash, a volume of wash solution approximating to half the volume of the cell suspension was added to the suspension. The exact PCV was then determined. The values for the PCV ranged from 65 to 80%. One volume aliquot of the cell suspension was then pipetted into isotonic sucrose (buffered with Tris–HCl to pH 7·4) to give a dilution of 1:10, the fraction of wash solution added together with the cells being taken into account. The actual volume of cells added was then determined by the PCV so that the result could be expressed on the basis of the actual volume of cells involved in the transport of cations. The mixture was incubated at 25°C in a water bath with very gentle shaking for various times. Because of difficulties in obtaining sufficient blood to complete this experiment on each patient, different individuals were used for studies carried out at the hourly intervals and at the minute intervals. After each incubation period the cell suspensions were centrifuged at 1000 *g* for 10 min, the sucrose supernatants separated from the cells and sodium and potassium concentrations were then determined on these supernatants as described.

Active and passive permeability of Hb AA and Hb SS erythrocytes

Active erythrocyte sodium transport was studied in three normal healthy Negroes with haemoglobin AA and in four sickle-cell patients (Hb SS). The cells were stored at 4°C for 2 weeks in a high sodium loading medium, which permitted them to gain sodium and lose potassium. The composition of the loading medium and the preparation of the sodium-loaded cells for the transport study were exactly as described by Post & Jolly (1957). For the transport studies, the sodium-loaded cells were incubated at 37°C for 4 h in two incubation media:

medium A contained 110·0 mmol/l KCl, 25·0 mmol/l Na_2HPO_4, 10·0 mmol/l glucose, 2·0 mmol/l $MgCl_2$ and 3·7 mmol/l adenosine; medium B was similar to medium A in composition but contained, in addition, 0·1 mmol/l ouabain and 1·0 mmol/l ethacrynic acid. The pH of both media was maintained at 7·4 with 1·0 mol/l HCl and the osmolalities were approximately 285·0 mosmol/l.

Incubation of cells in medium A permitted the measurement of total (active plus passive) efflux of sodium. Inclusion of ouabain and ethacrynic acid in medium B blocked the activity of both pumps I and II components of the erythrocyte sodium–potassium active pump (Hoffman & Kregenow, 1966), ensuring that the sodium efflux measured in this medium was entirely due to passive diffusion. Difference in efflux within the two media gave the net active sodium efflux.

In the performance of the transport study, stored cells were washed three times with $MgCl_2$–Tris–HCl buffer (pH 7·4). The supernatant of the third washing was shown to contain negligible or no sodium. The concentrations of sodium and potassium were determined on an aliquot of the resulting cell suspension. The remainder was reconstituted to a PCV of about 5% with the appropriate incubation medium. A portion (0·2 ml) of this suspension was added to 9·8 ml of the corresponding incubation medium in triplicate, mixed gently and placed in a water bath at 37°C for 4 h. The vibration of water produced by a stirrer in the water bath was adequate to ensure that the cells and media were well mixed. After incubation the cell suspension was centrifuged and the cells were separated. Experiments were deemed successful in those in which the supernatants were essentially haemoglobin-free; this was because active sodium efflux was evaluated by measuring the decrease in cell sodium as well as the increase in concentration of this cation in the incubation medium or supernatants. Sodium determinations were carried out as described above except that to avoid having to correct for intercellular fluid and/or cell volume changes, the sodium concentration was expressed as mmol/5 mmol of haemoglobin (Post & Jolly, 1957); 5 mmol of haemoglobin corresponds approximately to 1·0 litre of erythrocytes.

Microscopic studies

An important question in these experiments was whether the washing procedures and the exposure to iso-osmotic sucrose medium of low ionic strength made the sickle-cell erythrocytes sickle. Accordingly erythrocytes of both Hb AA and Hb SS subjects were examined in wet-mount preparations under a light-microscope. This microscopic examination was done after cells had been exposed to the $MgCl_2$–Tris–HCl buffer wash solution and also to iso-osmotic sucrose solution (1) under atmospheric pressure conditions, i.e. Po_2 approximately 21·4 kPa (160 mmHg), (2) under oxygenated ($O_2 + CO_2$, 95:5) and (3) under deoxygenated ($CO_2 + N_2$, 95:5) conditions.

For electron-microscopic observations, the erythrocytes were fixed in suspension in 15 g/l glutaraldehyde in 0·02 mol/l cacodylate buffer, pH 7·2, at 25°C under atmospheric conditions. After 2 h the cells were washed three times in the buffer and post-fixed in 10 g/l osmic acid in 0·1 mol/l phosphate buffer, pH 7·4, for 1 h at 4°C. The cells were dehydrated in suspension in graded ethanol and embedded in Epon 812. The sections were doubly stained with uranyl acetate and lead citrate. The significance of differences between means was assessed by using Student's *t*-test.

RESULTS

Erythrocyte sodium, potassium and water content

The distribution of erythrocyte sodium and potassium content in control subjects (Hb AA) and sickle-cell anaemic subjects (Hb SS) is shown in Fig. 1. The mean erythrocyte sodium concentration (±SEM) was 13·96±0·76 and 20·62±0·92 mmol/l of cell water in the Hb AA and Hb SS cells respectively. The difference between these mean values was highly significant

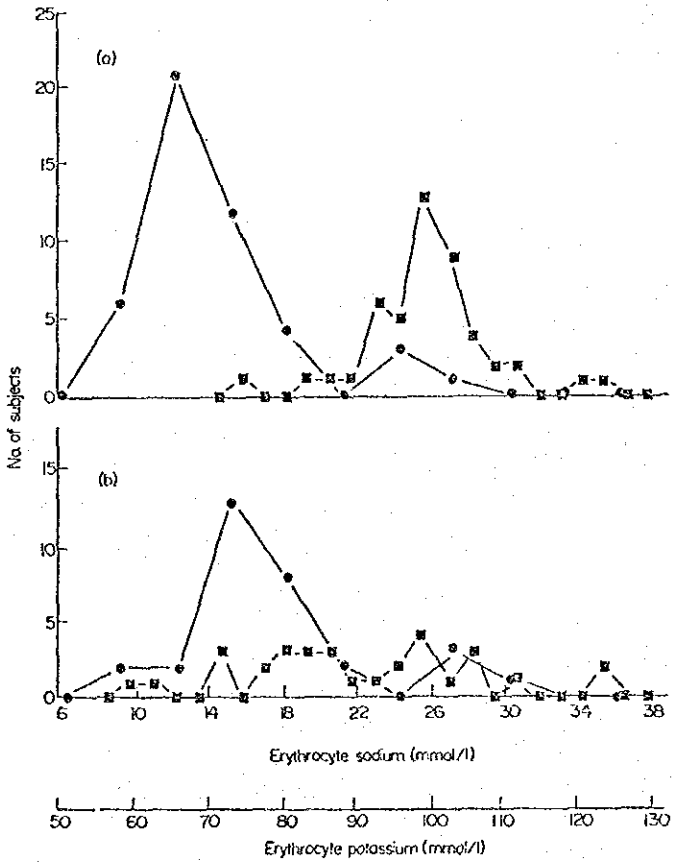


FIG. 1. Distribution of erythrocyte sodium (●) and potassium (■) (mmol/l of cell water) in (a) forty-seven normal Ghanaian subjects (Negroes) with Hb AA cells and (b) thirty-one sickle cell anaemic patients with Hb SS cells.

($P<0·001$). Measurement of erythrocyte potassium showed that the mean potassium concentration for the Hb AA cells was 99·07±1·19 (±SEM) mmol/l and that for the Hb SS cells was 89·37±2·82 (±SEM) mmol/l of cell water. The difference between these mean values was also significant ($P<0·05$). The mean values for cell water were 66·85±0·46 (±SEM) and 66·27±0·55

(±SEM) % (w/w) of cell for the two cell types respectively. The difference between the means was not significant ($P > 0{\cdot}5$). This confirms that the difference in erythrocyte sodium and potassium could not simply be the result of a change in cell water since only as much as 50–100% difference in cell water could cause a change in cell sodium concentration, as pointed out by Smith & Samuel (1970).

Sodium concentrations of erythrocytes of a small number of Caucasians showed marked differences from that of negroid Ghanaians. The mean sodium concentrations of Caucasian erythrocytes was 9·10±0·70 (±SEM) mmol/l of cell water. There was, however, no difference in the potassium concentration, the mean value being 95·34±1·47 (±SEM) mmol/l of cell water. These values compare very well with those reported in the literature. Cell water was not determined for the Caucasians.

The trapped plasma in these studies averaged 2·1% in the negroid Hb AA normal subjects and 1·6% in the Hb SS patients, showing that the inter-erythrocyte trapped water was of the same order of magnitude in both cell types (Table 1). Thus it was mathematically unnecessary to make special correction for the trapped inter-erythrocyte fluid when utilizing the PCV value for calculating the erythrocyte cation concentrations.

In all the subjects studied measurements were made in triplicate on the same occasion. The results did not vary by more than 2–5%. No differences were found in the sexes. Details of these findings are presented in Table 1.

Cation permeability of Hb AA and Hb SS erythrocytes in sucrose medium

Comparison of the efflux of potassium from Hb AA and Hb SS erythrocytes suspended in sucrose medium of low ionic strength revealed marked differences. Fig. 2(a) shows results typical for cells of six sickle-cell patients and six normal control subjects. The time-course of efflux of potassium from Hb AA erythrocytes was characteristically linear whereas that from the Hb SS cells was curvilinear. The initial rates of potassium efflux of the Hb AA and Hb SS cells were 0·19 and 1·30 mmol/min per 1 ml of erythrocytes respectively. The efflux of potassium in Hb SS cells declined markedly in the second hour and tapered off by the third hour. In another pair of subjects in whom the efflux of potassium was determined at shorter time-intervals, it was clear that initial rate of potassium efflux was still faster (Fig. 2b). This was 0·63 and 2·50 mmol/min per 1 ml of erythrocytes in the Hb AA and Hb SS cells respectively. The efflux of potassium in the latter sickle-cell patient began to decline by the end of the first hour. The two results shown in Fig. 2 illustrate the quantitative variation observed in these experiments and Table 2 compares the initial efflux rates for potassium for each member of the two groups studied. It is clear from the table that the initial efflux rate was four to thirty times greater in the Hb SS than in the Hb AA erythrocytes when each pair of subjects are compared. The rates were calculated from the slopes of the efflux curves occupying the first 10 min of the incubation period. We consider the initial rates as approximating to the true transport of potassium by diffusion or a 'passive' process, in as much as the external potassium concentration has not yet built up during this period to influence its continuous efflux. Thus it is inferred that the permeabilities of the Hb AA and Hb SS erythrocyte membranes suspended in sucrose media of low ionic strength must be characteristically different.

Active and passive sodium transport in Hb AA and Hb SS erythrocytes

The concentrations of sodium and potassium in erythrocytes stored in high-sodium medium

TABLE 1. *Sodium, potassium, water content and trapped inter-erythrocyte plasma (in packed cells for PCV determinations) in the blood of Hb AA and Hb SS subjects*

Intra-erythrocyte sodium and potassium are denoted Na_i and K_i, and plasma Na_o and K_o. Concentrations are in mmol/l of cell water or plasma. Cell water is as a percentage for 1·0 g of cells. Trapped plasma is as a percentage. *N* = Number of subjects; SEM = standard error of the mean.

Subject	Statistics	Intra-erythrocyte concentrations (mmol/l of cell water)			Cell water (%)	Plasma concentrations (mmol/l of plasma)		Trapped plasma (%)
		$[Na^+]_i$	$[K^+]_i$	$[Na^+]_i+[K^+]_i$		$[Na^+]_o$	$[K^+]_o$	
Normal Ghanaians (Hb AA)	*N*	47	47	47	11	47	47	11
	Mean±SEM	13·96±0·76	99·07±1·19	110·71±1·19	66·85±0·46	140·11±0·13	4·41±0·13	2·1±0·3
	Range	9·40–27·21	74·10–124·10	103·00–149·40	64·63–69·92	135·00–152·00	3·41–5·60	0·1–3·0
Ghanaian sickle-cell patients (Hb SS)	*N*	31	31	31	31	31	31	11
	Mean±SEM	20·62±0·92	89·37±2·82	109·36±2·54	66·27±0·55	143·00±4·72	4·63±0·08	1·6±0·09
	Range	10·90–34·30	61·60–124·00	87·10–141·20	63·97–70·55	136·00–150·00	3·80–5·40	0·1–3·0
Normal Caucasians (Hb AA)	*N*	8	8	8		8	8	
	Mean±SEM	9·00±0·70	95·34±1·47	104·43±1·64		144·25±81	4·35±0·10	
	Range	7·00–12·50	92·00–101·00	900·00–112·00		137·00–154·00	3·90–4·80	
P values								
Normal Negroes vs normal Caucasians		<0·0005	<0·05	<0·001		<0·05	>0·5	
Normal Negroes vs sickle-cell patients		<0·0005	<0·005	>0·5	>0·5	>0·5	<0·05	>0·5

at 4°C are shown in Table 3. This result shows that these erythrocytes retain an appreciable portion of the initial sodium load to which they were exposed, after they were subjected to subsequent washing. Therefore it is entirely feasible to use such sodium-loaded cells to study the characteristics of release of this trapped cation from the erythrocytes.

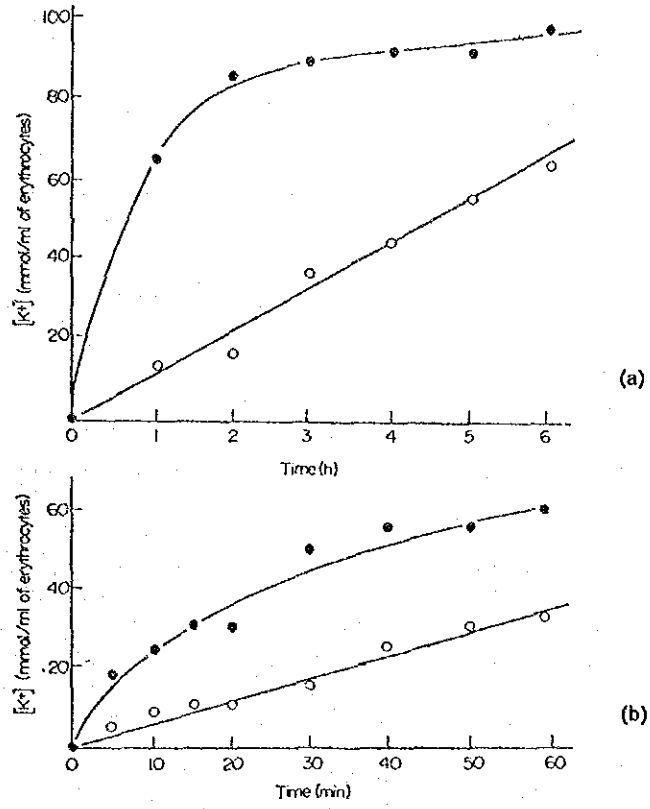


FIG. 2. Efflux of potassium ions from ○, normal (Hb AA) and ●, sickle-cell (Hb SS) erythrocytes suspended in buffered iso-osmotic sucrose solution (pH 7·4) at 25°C. (a) and (b) show results from two different patients studied on different occasions (see Table 2).

When the erythrocytes were suspended in medium A (see the Materials and Methods section) the efflux of sodium was markedly stimulated. The addition of ouabain and ethacrynic acid (medium B) inhibited this stimulation. Table 4 shows the active sodium transport obtained by simultaneous measurement of the rate of disappearance of sodium from the erythrocyte and its appearance in the incubation medium. There is a close correlation between these two mean values for the control cells, 26·6±4·7 and 25·9±3·2 (±SEM) mmol/4 h per 5 mmol of Hb respectively; and also for the sickle-cell erythrocytes, 61·9±3·6 and 56·9±5·9 (±SEM) mmol/4 h per 5 mmol of Hb respectively. Active sodium efflux was approximately twice as great in the sickle-cell erythrocytes when compared with the control cells. The difference between the mean

TABLE 2. *Comparison of the rates of initial efflux of potassium from Hb AA and Hb SS erythrocytes into iso-osmotic sucrose medium of low ionic strength at 25°C*

Note the variation in the rates of potassium efflux in erythrocytes from different subjects. The rates were calculated from the slopes of the efflux curves occupying the first 10 min of the incubation period. The complete results of experiments on subjects 1 and 6 are presented in Figs 2(a) and 2(b) respectively.

Subject no.	Rate of potassium efflux into iso-osmotic sucrose medium (mmol/min per ml of erythrocytes)	
	Hb AA erythrocytes	Hb SS erythrocytes
1	0·19	1·30
2	0·18	1·60
3	0·17	1·00
4	0·14	0·41
5	0·10	3·00
6	0·63	2·50

efflux values was statistically significant ($P<0·0005$). Another important fact that emerges from Tables 3 and 4 is that the active transport of sodium is faster in Hb SS cells even when the sodium contents of sickle-cells and normal cells are the same. The mean active sodium efflux obtained for Hb AA cells is comparable to the values reported by Post & Jolly (1957) obtained by the same method. Since the effect of ouabain in inhibiting the release of sodium is essentially equivalent to the absence of potassium from the medium, the ouabain-sensitive portion is taken as the active transport component of the sodium efflux, i.e. the pump flux.

For the measurement of passive sodium transport (that is ouabain-insensitive efflux) changes in the concentrations in the medium were more consistent and hence reliable. Table 4 shows

TABLE 3. *Post-storage concentrations of cations in erythrocytes*

Subject	Concentration (mmol/l of cell water)			
	Control cells		Sickle-cells	
	Na	K	Na	K
1	87·0	30·0	79·5	43·2
2	50·0	50·0	40·2	69·6
3	70·4	41·6	87·5	47·5
4	—	—	77·0	45·4
Mean ±SEM	69·1±10·7	40·5±5·8	71·1±10·5	51·4±6·1

P values (Hb AA vs Hb SS): Na>0·5; K>0·25

that the mean passive sodium transport in Hb AA and Hb SS cells was 1·5±0·2 and 3·1±0·2 (±SEM) mmol/4 h per 5 mmol of Hb respectively. The difference between these means was highly significant ($P<0·001$). The difficulty in obtaining comparable results for erythrocyte sodium changes might be due to the relatively small values measured and the washing procedure used to rid the cells of extracellular cations.

TABLE 4. *Summary of data on active and passive sodium transport in erythrocytes of three normal subjects (Hb AA) and four sickle-cell patients (Hb SS)*

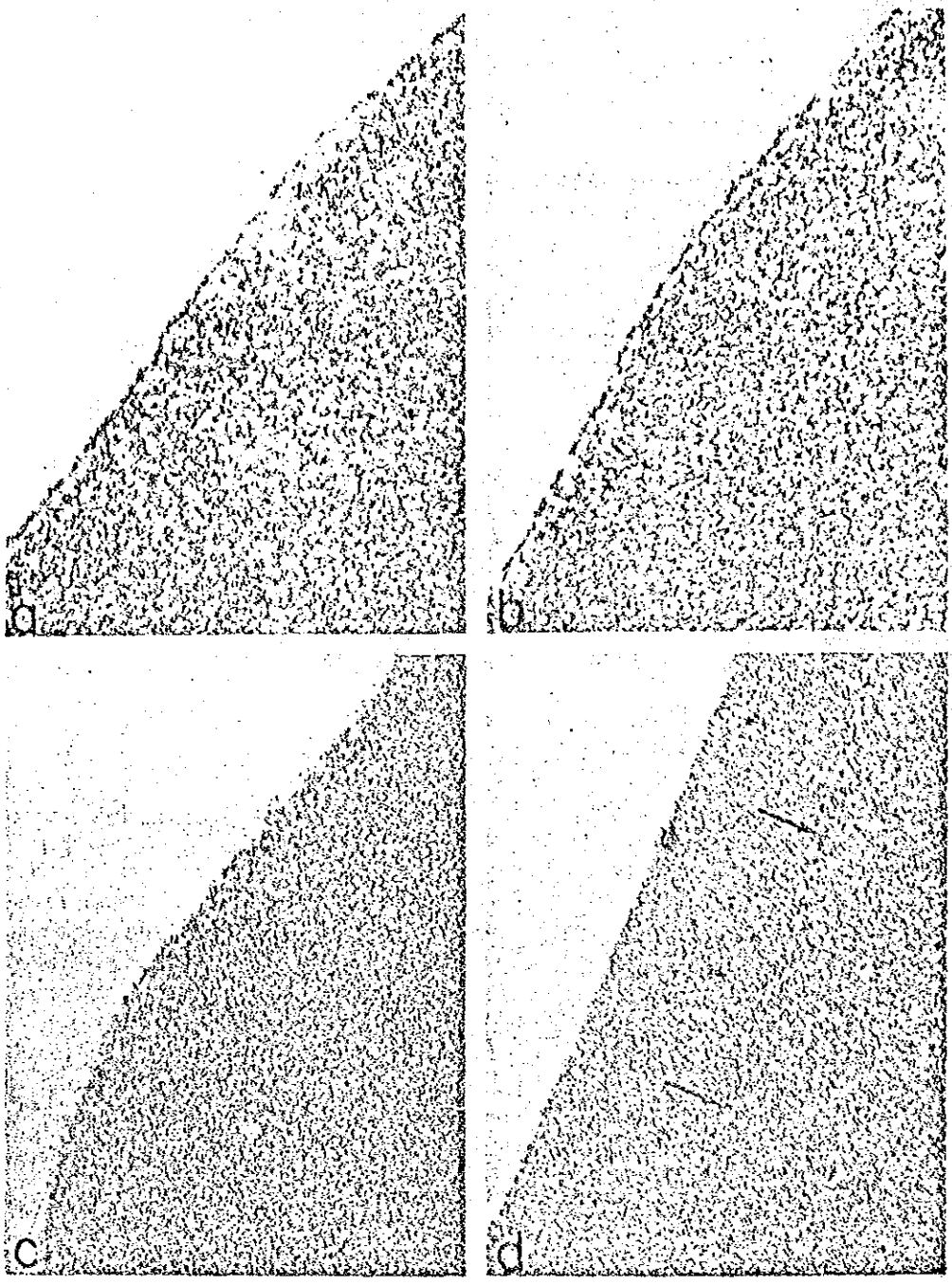
The study was performed on the same subject on two separate occasions represented as Expts. 1 and 2. The values are expressed as mmol/4 h per 5 mmol of Hb. The mean ±SEM (standard error of the mean) represents values for the two occasions.

Subject no.	Control cells (Hb AA)						Sickle-cells (Hb SS)					
	Active				Passive		Active				Passive	
	Medium		Erythrocyte		Medium		Medium		Erythrocyte		Medium	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
1	33·0	32·3	31·5	33·5	1·7	1·8	45·0	45·0	55·0	58·2	3·2,	2·6
2	27·0	27·0	36·3	34·3	2·3	1·6	60·0	50·0	61·3	66·5	3·4	3·3
3	25·0	11·0	12·0	12·0	0·8	0·8	47·5	71·5	53·3	85·0	2·6	3·5
4	—	—	—	—	—	—	91·0	45·5	56·8	59·2	3·5	2·9
Mean±SEM	25·9±3·2		26·6±4·7		1·5±0·2		56·9±5·9		61·9±3·6		3·1±0·2	
P values (Hb AA vs Hb SS)	Active: Medium <0·0005 Erythrocyte <0·0005										Passive: <0·001	

Microscopic studies

Erythrocytes examined in wet-mount preparations showed that more than 96% were present as biconcave discs in the Hb AA blood. The erythrocytes from Hb SS blood were composed of about 90% biconcave discs, 5% oval form and 3% sickled cells both when the cells were exposed to O_2+CO_2 (95:5) and to atmospheric pressure conditions. Moreover, the processes of washing and suspension in iso-osmotic sucrose did not provoke sickling in the Hb SS cells. However, when Hb SS blood was kept under deoxygenated conditions, 95% of the erythrocytes were transformed into holly-leaf, oval, oat and sickle shapes. Upon reoxygenation, about 85% of the cells were restored to biconcave discoidal form. The electron-microscopic studies on the

FIG. 3. Cell membranes in profile. (a) Portion of a normal (Hb AA) human erythrocyte fixed with glutaraldehyde and sectioned, showing a well-defined membrane tightly associated with the cytoplasm. (b) Type I Hb SS erythrocyte showing the membrane interrupted at several sites, which might suggest its porous structure. Some gaps are found between the membrane and the cytoplasm. (c) Type II Hb SS erythrocyte, in which the membrane is not detectable and the cytoplasm is fine-grained and dense. (d) Type III Hb SS erythrocyte: the outer surface is enveloped with a layer of haemoglobin extruded from the interior of the cell. Arrows indicate the membrane. (Magnification ×180 000.)



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The results of the permeability studies on normal and sickle-cell erythrocytes suggest that the permeability characteristics of the latter cells have been altered. The differences in potassium leakage observed between the two cell types may be due to the driving electrochemical gradient, fixed positive charges in the membranes and the possible reversible transition in membrane structure that occurs at low ionic strength (Passow, 1969; LaCelle & Rothstein, 1966). With regard to the influence of electrochemical gradient on potassium efflux, it is noteworthy that Arthur, Kurantsin-Mills & Addae (1973) have observed that the membrane potential of Hb SS erythrocytes is −18·16 mV whereas that of Hb AA cells is −10·31 mV. Thus, as pointed out by LaCelle & Rothstein (1966), such a high electrical driving force tends to increase cation efflux in a non-electrolyte medium of low ionic strength. The higher rate of passive diffusion of sodium from the pre-loaded cells in Hb SS erythrocytes compared with Hb AA cells suggests the existence of a 'leaky' membrane. This 'leakiness' has probably resulted from the sickling–unsickling cycles which the sickle-cells experience during their life-span, and may be present in both the sickled cells as well as the unsickled biconcave Hb SS erythrocytes.

The higher active sodium efflux observed in the Hb SS cells, even when their sodium content was the same as Hb AA cells, suggests a sodium–potassium active pump with a greater maximum velocity. The higher active sodium pump in Hb SS erythrocytes is consistent with the report that these cells have about twice the glucose consumption rate and lactate production rate of Hb AA erythrocytes (Tosteson *et al.*, 1955). Since the rate of energy production in erythrocytes is partly controlled by the magnitude of active sodium–potassium transport (Whittam & Ager, 1965), it appears that in order to maintain a balance in relation to erythrocyte trans-membrane cation distribution, the active cation transport in the Hb SS cells is enhanced to cope with the augmented passive diffusion of cations, thus establishing a different trans-membrane equilibrium for these cations.

It is also noteworthy that, as in the Hb SS erythrocytes reported here, erythrocyte cation hyperpermeability has been reported in other haemoglobinopathies such as heterozygous Hb Köln, Hb H disease and beta-thalassaemia (Jacob, Brain, Dacie, Carrell & Lehmann, 1968; Nathan, Stossel, Gunn, Zarkowsky & Laforet, 1969). It is at present not clear whether this abnormality is an intrinsic membrane defect or an aberration associated with the haemoglobin.

The biconcave appearance of the Hb AA and Hb SS erythrocytes in the light-microscopic studies strengthens the rationale that it is entirely reasonable to compare the two cell types, as is done in the present work. The wet-mount preparations revealed that most of both cell types were biconcave discs. Thus the Hb SS cells preserved the normal morphological characteristics when they were washed with the $MgCl_2$–Tris–HCl buffer for intracellular cation determinations, and also when they were suspended in sucrose medium of low ionic strength for cation-permeability studies. The fact that the Hb SS erythrocytes could be sickled by deoxygenation and unsickled by reoxygenation is consistent with the view that over 85% of these cells were not the irreversible sickle type (Bertles & Dobler, 1969). On the other hand, the permanent deformation of some of the sickle-cells due to the polymerization of haemoglobin results in the membrane aberrations seen in these studies. The undulating serrated margins and the attenuated shapes of these cells support the view that the membranes of these erythrocytes have suffered morphological damage, which has led to functional aberrations.

The results of the electron microscopy on the Hb SS cells show the possible types of erythrocytes in the peripheral circulation of the sickle-cell patient. The differences observed in the

electron micrographs of the Hb AA and Hb SS erythrocytes add support to the recent stereoscan electron-microscopy report of Lessin *et al.* (1972). These authors demonstrated that polymerization of Hb SS into helical rods produce tenting of the erythrocyte, resulting in fragmentation of the membrane. The erythrocyte loses portions of the membrane, which results in a cell with increased haemoglobin concentration, increased density and decreased resistance to osmotic and mechanical stress. As revealed by the ultra-thin sections of this study, sickle-cells show alterations in membrano-cytoplasmic relationships. These observations are consistent with the results of the permeability studies. Cell membrane distortions could alter trans-membrane distribution of sodium and potassium and this might subsequently affect the active and passive transport of these cations in the sickle-cell erythrocyte.

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Inhibitory substance(s) against AHC virus in human sera.
Clinical Virology, 2, 39-44, 1974.

[I-17]　AHCウイルスに対する阻止物質

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われわれはAHCウイルスに対するガーナ人および日本人血清中の中和抗体価を測定中[1]，中和時間の延長によって中和抗体価が著しく上昇することを観察した．この現象の本質を明らかにするため，種々の角度から検討した結果，正常ヒト血清成分中に，中和抗体それ自身とは明らかに異なる性質を有するが，AHCウイルスの増殖を阻止するある種のユニークな阻止物質の存在することが示唆された．今回は，このAHCウイルス阻止物質について，これまで得られた知見について簡単に要約する．

実験に供したAHCウイルスは，国立予研の甲野博士より分与をうけたYC-71-670株[2]で，MK細胞に一度増殖させてから用いた．中和は37°Cで所要時間行ない，ついで4°Cに1夜おき，残存ウイルス活性を試験管法によるCPEの出現，または密栓ボトル法によるプラック計測によって測定した．この時の培養温度は34°Cで行ない，測定に用いた細胞はすべてヒト胎児肺細胞（HEL-11, 12）であった．

ヒト血清中のAHCウイルスに対する中和抗体価は，表1に示すように，37°Cにおける中和時間を数時間に延長することにより，著しく上昇したが，モルモット免疫血清（甲野博士より分与をうく）では30分で中和が完了し，中和時間を数時間に延長しても抗体価の異状な上昇はみられなかった．対照のために行なったポリオウイルスに対する中和抗体価は，中和時間の延長によりほとんど影響されなかった．

このように中和時間の延長によって抗体価が上昇することを，プラック減少法によって反応速度の面から検討を加えた．すなわち，1：100

表1　AHCおよびポリオウイルスに対する中和抗体価の測定値におよぼす中和時間の影響

ウイルス	血清番号	37°Cにおける中和時間*							
		30分	1時間	2時間	4時間	6時間	8時間	14時間	24時間
AHC（YC-71-670株）	1876	0**	0	0	0	4	128	2,048	1,024
	1825	0	0	0	256	128	256	4,096	8,192
	1672	0	8	8	64	128	2,048	2,048	32,726
	モルモット免疫血清	512	512	512	1,024	1,024	2,048	2,048	2,048
	ウイルス対照（TCD_{50}）	NT	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	$10^{2.0}$	$10^{2.0}$	$10^{1.5}$	$10^{1.5}$
ポリオI型（Mahonney株）	1715	0	0	0	0	0	0	NT	NT
	1665	16	32	32	32	32	32	NT	NT
	2396	8	4	8	8	8	8	NT	NT
	1688	128	64	64	128	64	128	NT	NT
	ウイルス対照（TCD_{50}）	NT	$10^{2.5}$	$10^{2.5}$	$10^{2.0}$	$10^{2.0}$	$10^{2.0}$	NT	NT

*：37°Cにおける所要時間中和後，4°Cに1夜おき，ヒト胎児肺細胞に接種し，34°Cで培養，4日後に50% CPE阻止として判定．

**：表中の0は中和抗体の測定値が≦1：2であることを示し，その他の数字はタイターの逆数を示す．NTは測定しなかったことを示す．

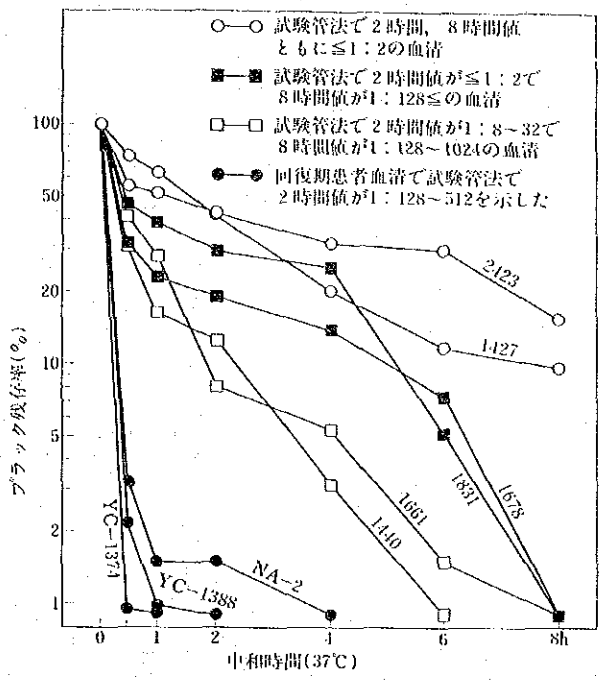


図1　ヒト正常および回復期患者血清によるAHCウイルスの不活化速度
1：100に稀釈した各被検血清に等量のウイルス液（120 PFU/0.1 ml）を混和し37°Cで所要時間（30分～8時間）中和し，残存ウイルスをプラック法により測定．プラック残存率は，各反応時間におけるウイルス対照のプラック数に対する100分率で求めた．

に稀釈した被検血清と等容量のウイルス液(120 PFU/0.1 ml）を混和し，37°Cで所定時間反応させたのち，残存ウイルスによって生ずるプラック数を測定した．その結果，図1に示すように，真の中和抗体が含まれていると考えられるAHC患者の回復期血清は30分から1時間のうちにほとんど大部分のウイルスを不活化した．これにくらべて，正常ヒト血清によるAHCウイルスの不活化速度は全般的に遅く，残存ウイルスは数時間以上かかってようやく10%以下に達した．試験管法（50% CPE阻止による）で2時間値（中和時間2時間の場合の見掛上の中和抗体価）と8時間値（同じく中和時間8時間の場合の値）がともに1：2以下，すなわち陰性の場合でも，プラック減少法によれば，反応速度は遅いがウイルスの不活化が明白にみとめられた．これにくらべて2時間値と8時間値のいずれか一方，または両方とも陽性を示した血清によるウイルスの不活化速度は，多少速かったが，回復期患者血清のそれにくらべれば格段に遅かった．

反応速度の異なる2種類のウイルス不活化物質の存在が示唆されたので，ウイルス分離によって確認されたAHC患者の急性期および回復期の血清をSephadex G-200で分画し，その各分画について50% プラック減少法によって2時間値と8時間値を算出した．その結果，図2に示すように，急性期血清の第3ピーク（アルブミン分画）にのみ8時間で中和活性を示す物質の存在することが明らかとなった．第1ピーク（IgM分画）および第2ピーク（IgG分画）の2時間値，8時間値はともに陰性で中和活性はみとめられなかった．回復期患者血清では第1ピーク（IgM分画）に2時間値と8時間値との間に余り差のない活性物質（おそらく真の中和抗体）がみとめられた．第2ピーク（IgG分

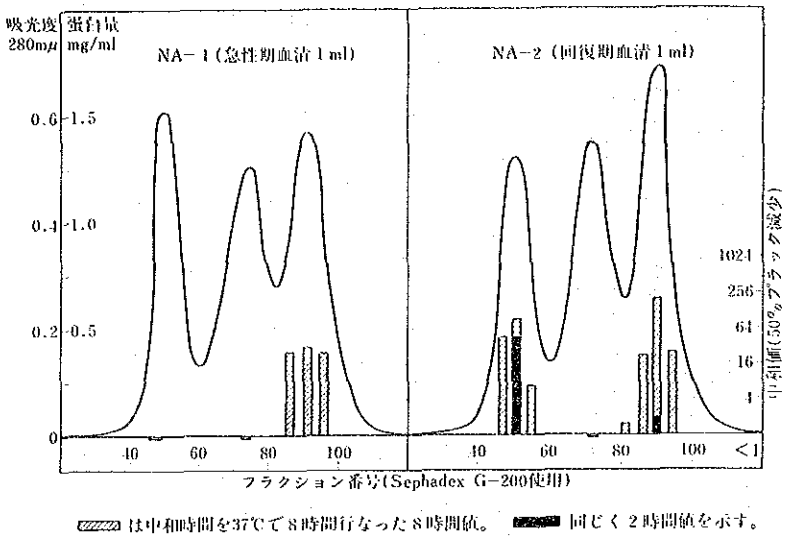


図2 AHC患者の急性期および回復期血清のSephadex G-200による分画とそのAHCウイルスに対する中和活性（カラムの溶出は0.85% NaClで行なった.）

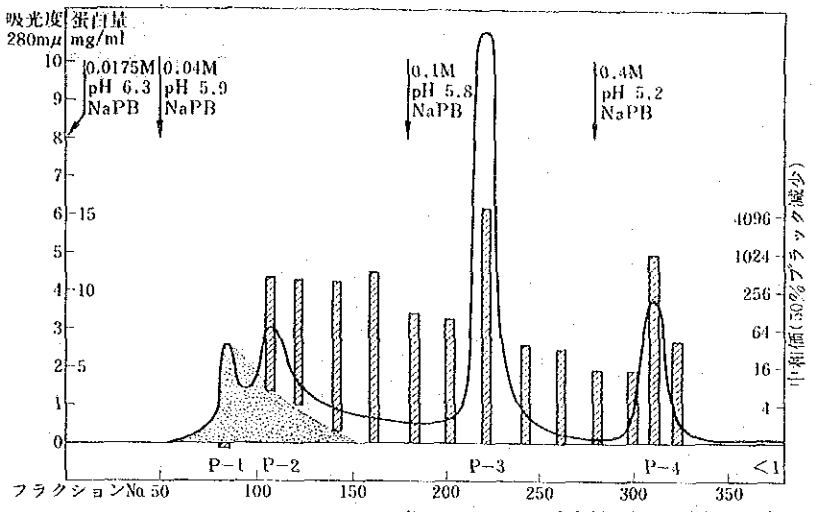
画）には活性がなく，第3ピーク（アルブミン分画）には8時間値で高い値を示す活性物質がみとめられた.

アルブミン分画に中和抗体とは反応速度の異なる増殖阻止物質の存在することがほぼ明らかとなったので，市販のアルブミン製剤について50% CPE阻止を示標とする試験管法によって，AHCウイルス増殖阻止作用の有無を検討した.すなわち，0.012～50 mg/ml の濃度範囲で，37°C 8時間中和させた場合の中和価（8時間値）を測定した.その結果，ヒトアルブミン（NBC, Cryst.×4, およびニチヤク）は共に0.048～0.78 mg/ml の範囲内で，ウシプラズマアルブミンFV (Armour) は0.048～12.5 mg/ml の範囲内でCPE阻止作用のあることがみとめられた.

アルブミン製剤中にもAHCウイルスの増殖を阻止する作用のあることが明らかとなったので，その中の1製剤（ニチヤクのヒト血清アルブミン）をDEAEセルローズカラムで展開し，その各分画についてAHCウイルスに対する阻止作用をプラック法によって検討した.その結果，図3に示すように，4つのピーク（P-1, P-2, P-3, P-4）が出現したが，P-1には阻止活性がなく，P-2は1：5.8から1：404の範囲内でのみ阻止活性がみとめられた. P-3は最も阻止活性が高く，1：4,940, P-4はそれにつぎ高く1：836のタイターを示した.

P-1には全く阻止活性がなく，P-2の低希釈度（1：5.8以下）でも阻止活性のないことは，これらP-1, P-2からフラクションNo. 160にかけて（図3の点々で示した部分），AHCウイルスの阻止作用を抑制する作用を有する物質の存在が予想されるので，これについて検討を加えた.すなわち，高い阻止活性を示すP-3をそれ自身は全く活性を示さないP-1（1 mg/ml）で希釈し，それにAHCウイルスを加え37°Cで8時間中和させたのち50%プラック減少率として中和価を求めた.表2に示すように，1：4,940の中和活性を示すP-3の値は，P-1の添加により1：23まで抑制され，P-1にはP-3のAHCウイルス阻止活性を抑制する効果のあることが明らかとなった.

以上のように，AHCウイルスに対する中和



▨ ; 各フラクションの中和活性(8時間値)を示す。
P−1からP−2にかけての点々の部分は中和活性抑制作用のある部分。

図3　ヒト血清アルブミンのDEAEセルローズによる分画とそのAHCウイルスに対する中和活性

ヒト血清アルブミン（ニチヤク）20%（w/v）液10mlをDEAEセルローズカラムにのせ矢印の溶液で溶出させた．NaPBはsodium phosphate bufferである．

表2　ヒト血清アルブミン製剤のDEAEセルローズカラム分画のAHCウイルスに対する阻止作用とその抑制作用*

分画（ピーク）	各稀釈におけるプラック残存率（%）							中和価
	1:1	1:4	1:16	1:64	256	1,024	4,096	(50%PR)
P−1	107.9	105.3	105.6	98.5	NT	NT	NT	<1:1
P−2	95.2	51.3	13.4	4.5	47.7	71.4	98.2	1:5.8~1:404
P−3	0	0	0	0	0	0	49.5	1:4,940
P−4	0	0	0	1.3	38.8	50.7	NT	1:836
(P−3)+(P−1)**	NT	3.5	29.9	98.6	102.1	100.3	NT	1:23

* ：図3の説明参照のこと．

**：(P−3)を(P−1)で稀釈し，各稀釈液と等量のウイルス液（120 PFU/0.1 ml）を混和し37°Cで8時間中和し，残存ウイルスをプラック法によって測定．ウイルス対照（分画を加えない）を100として，残存率を求めた．NTは試験しなかったことを示す．

抗体価を測定する際，中和時間の延長によって抗体価（見掛上）が著しく上昇する現象は，正常ヒト血清のアルブミン分画中に存在するある種の阻止物質によるものであることが明らかとなった．さらに，アルブミン中には，この阻止作用を抑制する作用を有する物質の存在も示唆された．

この阻止物質の本態については，まだ不明の点が多いが，従来の阻止物質にくらべて，かなりユニークなものと考えられる．また，実用的にはAHCウイルスに対する中和抗体価の測定に際して，本ウイルスはこのような阻止物質に極めて感受性の高いことに留意し，得られた測定値の解釈は十分慎重にする必要のあることを

指摘したい.

文 献

1) 南一守, 紺野謙治：アポロ11病の病因について. 日本医事新報 2568, 32-34, 1973
2) Kono, R. et al.: Pandemic of new type of conjunctivitis. Lancet 1, 1191-1194, 1972

——討 論——

甲野 フロアーからのご発言をどうぞ

南(福島医大) AHC は1969年ガーナで最初に発生しましたが，そのウイルス学的検索は私達が行ないました.

Chatterjee らの発表した論文に私達の行なったデータが引用されています. これは失敗の歴史ですが，私達は1969年10月にガーナで急性期の患者15名をクリニックに集め，チャタルジーが自らスクレーピングを取り，私達がそれを直ちに HeP #2 と H-eLe, Vero に接種して3代継代しましたが全部陰性でした. 培養は30°C でした. 血清は完全なペアがとれましたので，15例についてアデノおよびヘルペスウイルスに対するCFを行ないましたが，いずれも上昇せず陰性でした.

日本で HeLa 細胞で分離されましたが，私達のもっていった HeLa 細胞は AHC ウイルスに対して感受性のないことが後で，甲野先生にいただいた670 株について調べて分りました。

もう一つ，68年と70年，71年にガーナで取った血清が幸い手元にありましたので，670株で中和抗体の保有率を検討しましたら，有意の上昇がありました。ガーナで Apollo 11として世界で初めて報告された AHC も，甲野先生の分離されたウイルスによって起こったことを強く示唆するデータが得られております。

山田(群馬中央病院) 現在までに分離された株から血球凝集反応に陽性な株は得られなかったでしょうか.

甲野 私も，徳田先生同様，一応やってみましたが陰性でした。

Minami, K., and Konno, K.:
Inhibitory substance(s) against AHC virus in human sera.
Clinical Virology, 2, 29 - 44, 1974.

During the course of virus neutralizing (VN) test against AHC virus, it was found that there were two kinds of VN substances in human sera. One was specific VN antibody, which was found in gamma globulin fractions and rapid reactive. The other was non specific VN substance (inhibitor), which was demonstrated in albumin fractions and slow reactive.

In the free discussion, an episord was presented on the first trial of AHC virus isolation in Ghana in 1969 with an unsuccessful end.

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[I-18] ガーナの飲料水と下水から分離したウィルス

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(Department of Microbiology, University of Ghana Medical School)

OTATUME, S. and ADDY, P. A. K.:

Viruses Isolated from Drinking Water and Sewage in GHANA.

Medicine and Biology, 88, 89-94, 1974.

The authors attempted to isolate the viruses from drinking water in rural area and sewage in urban area, and obtained following results;

Twenty-one strains of viruses were isolated from 70 samples collected (virus isolation rate: 30%). Viruses were recovered from 10 samples out of 35 samples of drinking water (28.6%), 9 samples out of 16 samples of sewage (56.3%), 1 from bathing/washing water, and 1 from drinking water for cattle. So far tested, some of the isolates were identified as type B-3 coxsackievirus (6 from drinking water and 2 from sewage), type 1 poliovirus (1 from drinking water), type 7 echovirus (1 from sewage), and type 19 echovirus (1 from sewage).

(Authors' translation)

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ガーナの飲料水と下水から分離したウイルス

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熱帯地方の開発途上国においては，衛生施設，とくに上下水道の設備はまだ充実していない．西アフリカのガーナもこの例にもれず，首都アクラ市でさえ多くの家庭は上下水道の施設を持っていない[1,2]．さらに市外に出れば，下水道は無論，上水道設備もごく一部の地域にしか設置されていないのが現状である．村落部では多くの場合，自然の河川や池の水を濾過も殺菌も行なわないままで，飲料水として使用している．その水は多量の泥や各種の有機物を含み，水草が繁茂し，野生動物が住みついている湖沼もある．このような状態であるから，当然，水中には多くの微生物が浮遊しており，経口伝染病の病原微生物が存在することも十分予想された．著者らは1971年からガーナのアクラ市周辺地域の飲料水を集めて，その中のウイルスの分離を試みた．また都市部の下水等も採取して，同様に検査を行い，熱帯地方の飲料水および下水中におけるウイルスの分布状態を観察した．

材料と方法

飲料水（人間用）および雑用水（水浴，洗濯用水）はアクラ市周辺の村落部から集め，下水はアクラ市内から集めた．家畜用飲料水はアクラ周辺の家畜（主に牛）放牧地区の水を採取した．採水は表層の水草や底のドロを混ぜないようにして汲み上げ，2-3lずつ大型びんに入れた．河川の場合は流れの早い方から，池の場合も出来るだけ中央に近い方から汲み上げた．採水は主に午前中に行い，水温，pHを測定した上で実験室に運搬した．

採取した水はまず3,000回転15分間遠心して粗大なゴミなどを除き，次いで13,000回転で60分間低温で遠心し微細沈澱物を除いた．最後に40,000回転2時間超遠心を行い，生じた沈降物を $1/20$-$1/30$ 量のPBSに浮遊し，これを接種液とした．ウイルス分離は主にHEp 2細胞を用い，前記の接種液の0.1 ml-0.2 mlを中試験管に培養したHEp 2細胞に接種した．3-4代継代して細胞変性陽性のものを各種の生物学的性質を調べた上で，WHO[3]のエンテロウイルス用プール血清でウイルスの同定を行った．

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成績

1. 調査地域および採取標本の概況

対象地域は首府アクラ市を中心とする半径40 km の地区で大部分は Greater Accra Region 内である．この附近は標高20–100 m の Accra Coastal Savannah (High grass savannah) で一部は Akwapim Scarp に接している．都市部，海岸地帯，草原地帯，森林地帯に大別されるが，それら区分は画然としたものではない．雨量は年間70–100 mm で，山地（森林地帯）では110–140 mm 程度に達する．アクラ市内は別として，村落部の人口密度は20–26/km² (1960)[4] である．農業従事者が大部分で，漁業（海岸部）や林業（山地）に従事するものもいる．

表 1 ウイルス分離状況

用途	標本数	分離陽性例数	百分率
飲料水（人間用）	35	10	28.6
飲料水（家畜用）	14	1	7.1
雑用水（浴・洗濯用）	5	1	20.0
下水	16	9	56.3
合計	70	21	30.0

表 2 飲料水からのウイルス分離状況

水源の状態		標本数	分離陽性例数	百分率
河川	幅2 m以上	9	4	44.4
	幅2 m以下	2	0	—
湖沼		22	6	27.3
井戸		2	0	—
合計		35	10	28.6

標本として採取した水は合計70で，内訳は飲料水（人間用），35，同（家畜用），14，下水：16，雑用水5である．これらの水の一般的状態は非常に濁っており，飲用にはもちろん，雑用水としても不適当と思われるものが多かった．全ての湖沼の水は多量の土壌成分，有機物，水棲微生物を含んでいた．河川の中には比較的きれいな水の得られるところもあり，また2カ所の湧水井戸はともにやや良質の水を出しておった．水温は26–31°C 程度であったが，中には34°C という高温のものもあった．pH は5.5–7.8の範囲内であった．

2. ウイルス分離状況

表1に示したように村落部の飲料水から28.6％の割合でウイルスが分離された．表2に示すように，飲料水のうちでは河川の方が池や沼よりはウイルス分離

率が高いが，井戸や水源に近いところの小川の水からは1例も分離されなかった．一方都市部の下水からは56.3％の高率でウイルスが分離された．下水のうちの2カ所については毎週1回ずつ6週間にわたり水を採取したが，両方とも毎回ウイルスが分離された．家畜用飲料水は家畜由来のウイルスが多いと予想され

表3　ウイルス分離陽性例の採水場所，日時，用途，ウイルス型および地理的関係

標本番号	採水場所	採水月日	水の用途	ウイルス型	備考
1	Oblogo	26/10/'71	D	Cox. B-3	Weija 川，Accra 市水道の水源
3	Botianaw	〃	B & W	ND	海岸付近の小川
4	〃	〃	D	Cox. B-3	3の上流
5	Aplaku	〃	D	Cox. B-3	1の下流
8	Dankyira	6/11/'71	D	Cox. B-3	Tokuse-Nsuobri 街道添いの部落
11	Obakrowa	〃	D	ND	〃
13	Pokosea	8/11/'71	D	ND	1の上流
16	Manhia	〃	D	ND	1と13の中間の沼沢地
19	Akramanman	〃	D	Polio-1	Savannah 内の孤立村落
20	Obom	15/11'71	D	Cox. B-3	8と同じ街道沿いの部落
21	Oshia	〃	D	Cox. B-3	〃
39	Accra	6/12/'71	S	Echo-7	Accra 市の中心部
40	〃	〃	S	Echo-19	〃
41	〃	〃	S	Untyped	〃
42	〃	〃	S	Cox. B-3	〃
49	Nunga	31/ 1/'71	D-C	Polio(U)	Accra 市郊外(海岸部)の村落付近
51	Accra	2/ 2/'71	S	Cox. B-3	Accra 市郊外(草原地帯)
52	〃	〃	S	Cox. B-5	Accra 市の中心部
53	〃	〃	S	ND	〃
69(1-6)	〃	24/9-29/10/'71	S	ND	〃
70(1-6)	〃	〃	S	ND	〃

註　D；飲料水，　B & W；雑用水，　S；下水，　D-C；家畜用飲料水，
Cox.B-3；コクサッキーウイルス B-3型，　Polio-1；ポリオウイルス1型
ND；未同定，　Echo-7；エコーウイルス7型，　Untyped；同定不能，
Polio (U)；ポリオウイルス型未定．

たが，実際には最も低い分離率にとどまった．雑用水は例数も少なかったが，ウイルス分離率も低かった．

3．分離ウイルスの型

現在までに同定されたウイルスは全分離株21株中14株である．コクサッキーB-3型 (Cox. B-3) が8株で最も多く，他はポリオ1型 (Polio-1)，Cox. B-5,

エコー (Echo- 7), Echo-19型が分離されたがいずれも1株ずつである．また，Polio ウイルスで型未同定のものが1例と WHO のプール血清で同定出来なかったものが1例あった．Cox. B-3のうちの6株と Polio-1は飲料水から分離されたものであるが，他は下水または家畜用飲料水から分離されたものであった．表3にウイルスが分離された場所と採水月日およびウイルスの型を示した．

4．ウイルス分離地点の地理的関係

これらのウイルスが分離された地点（とくに飲料水採取地点）の相互関係を見ると，同一水系 (Weija 川流域) に属すると思われるところが，標本 No. 1 (Cox. B-3)，5 (同)，13，15の4地点，また No. 3，4 (Cox B-3) は同一の川である．同じ道路に沿って点在する部落の採水池は No. 8 (Cox B-3)，No. 11，20 (Cox. B-3)，21 (同) である．No. 4と No. 5は別水系であるが部落間の距離は近く，相互の交通は頻繁である．表の備考欄に地理的関係を略記した．

考察

アクラ市は西アフリカの海岸ぞいの都市の中では比較的乾燥していると云われる．1967年の記録[5]によると年間の降雨日数は69日あるが雨量は83.5 mm である．雨期は4月から10月までで，そのうち4，5，6の3カ月間に年間雨量の半分以上が降っている．7，8月はやや雨が少なく，9月と10月に雨量が若干多くなる．11月から翌年の3月までが乾期で，雨はほとんど降らず，高温が続く．この時期には村落部の住民は水の確保に非常に努力をしなければならない．一般に飲料用水として地下水を利用している所は少なく，調査した範囲では山地で2例を見ただけで，他の33例はいずれも浅い池に溜った水や河川の水をそのまま利用しているにすぎなかった．このような飲料水が，なぜ，どのように汚染されているかについては現段階ではまだ未解決であるが，水源に近い小川や湧水からはウイルスが分離されなかったことから，汚染は地下水脈によるものではなく，表層において行なわれたものと考えられる．実際に彼ら現地住民の飲料用水池などは部落から非常に離れた位置にある場合が多く，これを合目的的に考えれば居住地周辺の排水や住民の排泄物の流入を防ぐための配慮と云えるかもしれない．それにもかかわらず，高率に腸管系ウイルスが分離されたことは住民が飲料用水について何か誤った取扱いをしているのではないかと考えられた．しかも，濃縮比や接種量から逆算すると，飲料水・下水中のウイルス濃度は100 ml 当り6またはそれ以上の感染性ウイルスと計算され，相当濃厚に汚染されていたことになる．この程度まで汚染が進むためには水量から考えても間接的な汚染ではなく，人または動物（家畜あるいは野生動物）[6]の排泄物が直接流入したと考えざるを得ない．動物の排泄物流入を防ぐためには，その動物の侵入を防ぐ処置を必要とするが，人間のそれについては衛生教育を徹底することによって防ぐことが出来るので，ウイルス流入の経過を追求してみれば，汚染を最小限に止めることが可能かもし

れない.

いずれにしても，飲料水中に存在する腸管系ウイルスが，やがてその地域の住民に感染を拡げて行くであろうことは容易に想像される．その例として，アクラ市近辺の3つの部落(いずれも飲料水からウイルスが分離された部落)の幼児から糞便を集め，腸管系ウイルスの分離を試みたところ，8月(雨期)は25％，3月(乾期)には16.4％からウイルスが分離された．特にそのうちのA部落では雨期に幼児の40％からウイルスが分離されたが，そのウイルスの大部分はエコー20型であり，乾期には28％で，その大部分がポリオ2型であった．しかし他の，人口の多い村では分離されたウイルスが特定の型によって占められるというような現象はなかった．またB部落では雨期は23％の幼児がウイルスを保有しておったが，6カ月後の乾期には水道が設置された関係か，7％のウイルス分離率に低下した(大立目，未発表)．

飲料水から Cox. B-3 が分離された村落のうち3村は1本の道路で連絡していたが，その道の一端はアクラ市から西へ伸びる幹線道路に接続しており，他の一端は鉄道沿線のやや大きい部落である．その地の住民が部落間をどの程度交流しているかは判然としないが，相互の距離は直線にして5-8 km 離れており，いずれも草原地帯，森林地帯の独立した部落で水脈等の連絡はなかった．従って，このウイルスが交通路に沿って伝播された可能性は十分に考えられる．ただし，それらの Cox. B-3 ウイルスが人以外の動物由来のものだとすれば，この可能性は根本から覆されるだろうし，必ず人由来であるとも云いきれない．

一方，Cox. B-3 型ウイルスがほぼ同時期に市内の下水からも分離され，相互の関連性を思わせられたが，市内の幼児から分離されたウイルスの中には1例も同ウイルスが見つからなかった点も問題である．

飲料水あるいは下水中に含まれるウイルスの分離に関して多くの研究[7]が発表されているが，熱帯アフリカにおけるこの種の研究はまだ十分ではなく，未解決の問題が多い．この水由来のウイルスを分離するには水中のウイルスの濃度が低い上に，いろいろな夾雑物の存在する中からそれだけを選択的に集めなければならないので，各種の技術的考案がされている．われわれの行なった遠心法も1つの方法であるが，ウイルスの濃縮比（40,000 rpm，2時間の遠心で沈殿するウイルスについて）は約 $1/30$ であり，Wallis[8] らのミリポアフィルターによる $1/1000$ という濃縮比よりは明らかに低い．従ってウイルス分離率も低いが，彼らの場合材料が下水であるのに対し，本実験の場合は主に飲料水である点は根本的に違うところである．さらに，このような低い濃縮比でも，かなり高い割合でウイルスが分離されたことはウイルスの分布，濃度などが予想以上に高かったということになろう．

下水中のウイルスについて取り上げられることは1971年の12月に市内の健康な幼児たちの糞便から Echo-19 と Echo-7 が多数分離された時期に，下水からも同型のウイルスが分離された点である．このことから下水中のウイルスを継続的に注意していれば，腸管系ウイルスの流行を監視する試みも不可能ではないだろ

う．ただ，実験成績が示すように，ある地点では常にウイルスが分離されたが，他の場所で必ずしも分離は成功しなかった．

最後に，根本的な問題として考慮しなければならないのは，この研究で分離されたウイルスが果して真に水由来のものであるかどうかということである．われわれがこれらを水由来のウイルスであるとした理由は次のような点からである．例えば Cox. B-3 が多数分離同定されたが，飲料水や下水を野外から採取し，実験室内で処理をした日時が違っていても同型ウイルスが検出されたこと，採水量は 2*l* 以上であり，採水器具により影響は少ないこと，実験期間中に市内では Cox. B-3 の流行はなく，実験室勤務者からウイルスが迷入したとは考えにくいこと，実験担当者を替えて，保存中の接種原液から再び行なった分離実験でも同型ウイルスが検出されたこと，などの諸点から，これらのウイルスは実験上の誤りで迷入したものではないと考えているためである．さらに同じ川の上流と下流とか，同じ道路上に点在する部落から同型のウイルスが分離された例 (No. 1 と 5，または No. 8, 20, 21) あるいは市内の幼児の間で流行したと同じ型のウイルスが下水からも分離された例 (No. 39, 40) のように微生物生態学的に説明つきうる形でウイルスが分離されているので，これらの成績を単なる実験の誤りであるとして棄却すべきではなく，今迄の常識[9]を越えるような飲料水の汚染があったものと解釈すべきである．今後，飲料水汚染問題は熱帯地方における衛生教育の課題となろう．

結論

ガーナの村落部の飲料水および都市部の下水からウイルスの分離を試み，次の成績を得た．

総計 70 例の標本から 21 株 (30 %) のウイルスが分離された．そのうち，飲料水から 10 株(28.6 %)，下水から 9 株(56.3 %)，家畜用飲料水から 1 株，雑用水から 1 株のウイルスが分離された．分離株のうち，これまでに同定されたものはコクサッキー B-3 型 (Cox. B-3) ウイルスが 8 例 (飲料水から 6 株，下水から 2 株)，ポリオ 1 型 (Polio-1) ウイルス (飲料水から分離)，Cox. B-5 ウイルス (下水から)，エコー 7 型 (Echo-7) ウイルス (下水から)，Echo-19 ウイルス (下水から) がおのおの 1 株ずつ同定された．

1) Otatume, S. & Addy, P. A. K.: Enteroviruses in infants in Accra, A preliminary report. *Ghana Med J* **12(3)**: 282-286 1973 —2) Acquah, I.: Accra survey. Ghana Univ. Press Accra 1972 58-59 —3) Schmidt, N. J., Melnick, J. L., Wenner, H. A., Ho. H. H. & Burkhardt, M. A.: Evaluation of enterovirus immune horse serum pools for identification of virus field strains. Bull W H O **45**: 317-330 1971 —4) Grove, D. & Huszar, L.: The towns of Ghana. Ghana Press Accra 1964 59-64 —5) Min. of Health, Gov. of Ghana: Medical statistical report No. 1. Ghana Publishing Corp. Accra-Tema 1967 —6) Kalter, S. S.: Picorna viruses in water, In Berg, G. (Ed) Transmission of viruses by the water route. JohnWiley N. Y. 1965 253-267 —7) Melnick, J. L. (Ed): International virology. S. Karger 1972 323-326 —8) Wallis, C. & Melnick, J. L.: Concentration of viruses from sewage by adsorption on milipore filter. *Bull W H O* **36**: 219-225 1967 —9) Mosley, J. W.: Transmission of viral disease by drinking water. In Berg, G. (Ed) Transmission of Viruses by the Water Route. John Wiley 1965 5-24

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[I-19] 最近のガーナにおける感染症の傾向

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OTATUME, S.:
Recent Trend of Infectious Diseases in Ghana.
Nettai (Tropics), 8, 149-158, 1974.

Various infectious diseases were prevailing in Ghana even now. Most important diseases of them were measles, infective hepatitis, tetanus and malaria. Tuberculosis and enteric fever were also serious. Many children suffered from pertussis and chickenpox. Though the number was limited, case fatality rate of yellow fever was quite high.

To prevent such infectious diseases in the now developing countries, it is necessary to embark on a nation-wide mass vaccination program. Besides, it should be also stressed to establish the infrastructural health service system, and to develop the education of public and personal hygine for the peoples. (Author's translation)

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Recent trend of infectious diseases in Ghana.
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最近のガーナにおける感染症の傾向

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1968年にガーナ政府が発表した統計[1,2]によれば，1967年の確定死亡者の約30%は感染症であった。特に5歳以下の幼児においては，この比率はもっと高く，死亡者の35～40%は各種の感染症と推定された。病院や保健所を訪れる一般外来患者の60%，入院患者の20%程度が感染症で占められていたという報告[3]もある。このように，本邦や欧米では非常に少くなった伝染性疾患が，今なお多数の犠牲者を出しながら，繰り返し発生しているのがガーナあるいは西アフリカ全般の現実である。

著者は1971年から1973年まで，海外技術協力事業団の委嘱を受け，医療協力専門家としてガーナ大学医学部の微生物学教室でウイルス学の研究指導に従事したが，この間に得た各種統計や記録を基に，最近4年間のガーナの感染症の動向をまとめてみた。引用した主な資料は GHANA Medical Journal に掲載された Epidemiological Notes (四半期ごとの厚生省発表患者発生届出記録) を集約したものである[4-10]。

これらの数値は主に各地区の医務官 (Regional Medical Officer of Health (RMOH) からの通報に依っている。もちろん，これがガーナの全地域，全住民をくまなく調査した成績とは言えない。ガーナにおいては基本的な人口動態統計さえも依然として未整備であり，疫学的調査に必要な他の諸条件も不十分である。加えて，住民側の社会衛生的な保健意識も低いので，正確な統計が得られ難いことは政府当局者自身も認めていた。このような不満足な統計であったが，最近では関係者の熱意によって徐々に通報システムが改善されつつあり，統計の信頼性も高まってきた。

本稿の目的は最近のガーナにおいてどのような感染症の届出が増え，あるいは減りつつあるかを見て，ガーナの感染症の動向の一端を紹介することにある。

ガーナの人口

前述したように，正確な統計ではないが1967年のガーナの総人口は814万人[2]と報告された。これは全国の42地区で行われている登録を基礎にして推計されたものである。毎年の推定人口増加率は2.6～2.9%になっている。この比率で増加した場合のガーナの総人口は1969年：859±2万人，1970：883±4万人，1971：907±5万人，1972：925±7万人となる。この算定の基礎となっている Gaisie, S. K.[11]の推定出生率は44～55，同死亡率は21～25である。また，全死亡者の10%は生後1週間以内，25%が同じく1年以内に死亡したと推定している。

ガーナの気候

海岸のアクラ市および森林部のクマシ市と北部のサバンナにあるタマレ市では気候にかなりの差がある[2]。最も気温が高いのはタマレ市で，2～3月の最高気温は40°C 以上に昇り，著しく乾燥する。クマシ市は比較的涼しく，雨量，降雨日数も多い。アクラ市は最も雨量が少い。雨期はクマシ，アクラでは6月をピークとする大雨期と10月ごろの小雨期と2回あるが，タマレでは9月をピークとする1回だけである。

ガーナの届出感染症

表1に示したように24種類の感染症が届出られている。これらの疾患に関する過去の記録は多くないが，今世紀の前半には各種の伝染病が猛威をふるっていた[12]。その中には関係者の努力によっ

Table. 1　Communicable diseases in Ghana (Notified during 1969-1972)

Diseases	1969		1970		1971		1972	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Cholera	0	0	3,815	252	11,965	570	625	32
Plague	0	0	0	0	0	0	0	0
Smallpox	0	0	0	0	0	0	0	0
Yellow Fever	0	0	12	7	0	0	4	4
Typhus	0	0	0	0	0	0	0	0
Relapsing Fever	0	0	0	0	0	0	0	0
Enteric Fever	2,116	75	2,157	104	4,202	120	3,926	146
Food Poisoning	357	25	567	4	561	4	415	1
Tuberculosis	6,667	137	4,721	104	5,605	100	5,091	67
Anthrax	10	2	132	20	22	2	39	9
Leprosy	822	10	953	24	933	22	981	9
Diphtheria	62	2	15	2	14	1	23	2
Pertussis	5,282	31	12,032	25	14,664	24	18,064	22
Tetanus	1,329	223	1,341	278	1,369	310	1,370	310
Poliomyelitis	134	2	111	1	129	3	156	7
Chickenpox	9,923	31	20,311	9	67,651	12	68,209	17
Measles	33,065	208	45,783	200	94,870	356	95,408	282
Infective Hepatitis	4,779	107	7,134	168	9,510	133	11,490	211
Rabies	61	54	29	29	18	18	24	24
Trypanosomiasis	49	0	129	0	158	3	126	0
Infectious Yaws	5,343	0	9,676	0	12,757	0	21,970	0
Cerebrospinal Meningitis	230	21	414	86	597	57	854	91
Ophthalmia Neonatorum	680	0	1,081	0	779	0	773	1
Puerperal Pyrexia	600	5	735	6	623	11	458	4

て全く姿を消したものもあれば，近年，著明に増加したもの，あるいは新しく侵入したものもある。前者の例は Plague, Smallpox, Relapsing Fever などであり，後者としては Chickenpox, Measles, Infective Hepatitis などのウイルス性疾患や Cholera などがあげられる。前述のようにガーナの気候風土に地域差が著るしいので，Cholera や Yellow Fever (Y.F.) のように海岸地方や森林地帯で多く流行するもの，髄膜炎 (C.S.M.) のごとく主に北部のサバンナ地帯で流行するものなど，患者の発生分布は必ずしも均一ではない。また乾期と雨期の差も激しいので，Chickenpox, Measles, C.S.M. のように季節的な流行パターンの見られるものもある。以下，各疾患について，その趨勢を述べて行く。

Cholera : 1970年の8月まで絶無であったこの疾患が同年9月ガーナに侵入した[13]。以来，数カ月の間に，海岸地帯の住民の間に猛烈な勢いで蔓延して，1971年の1月には毎週1200名以上の患者が発生した。関係者の懸命な努力によって[14]，以後は次第に減少したが，現在でも，海岸部の村落などには少数の患者が発生し続けているようであつた。ガーナの村落部においては，今でも，飲料水を川や池の泥水に求めている所が多く[15]環境衛生の立ち遅れが認められるので Cholera の流行に対しては極めて悪い条件にあったと言える。

Plague : 1926年以後は全く発生していない。1901年から1926年までの間に2回の流行があったが，1908年の流行が最大であった[12]。

Smallpox : 1901年から1968年の9月まで，年間100人前後の患者が発生した流行は多数あり，患者数1000人以上の流行も5回あった。この間，ワクチン接種は1920年頃までは毎年10万人以下に過ぎず，1948年頃になってようやく毎年100万人前

後に種痘が行われるようになり，それと共に患者発生の減少が目立ち始めた。1967年の2月から開始された天然痘および麻疹撲滅計画（USAID と西アフリカ18カ国との共同事業）によって，4年間に全住民の8割に種痘を行った結果，1968年10月の例[16]を最後にガーナでは全く天然痘は発生していない。近隣のマリでも同様な傾向が見られた[17]。

Yellow Fever（YF）：1970年に12名発症（死亡7名）し，1972年に4名発症(全例死亡)した。ただし，これらは確認された者だけで，実際はもっと多いとみられる。1972年に発生したうちの1例は孤立した農場内の発生であり，Jungle Yellow Fever と見なされた。今世紀に入ってから，発生患者数100名以上の流行が2回，10名以上の流行は何度もあった。患者の発生は森林地帯に多い。興味があるのは1927年以前の Y.F. の流行では致命率は高くなかったが，1928年，すなわち野口と Young(1928年の Y.F. はこの二人だけ）の死亡以後は致命率が非常に高くなったこと[12]である。

Tyhus：1901年以来，現在に至るまで，ガーナでは本疾患が流行した形跡は全くない。ただし，各種の血清反応を行って見ると，抗体陽性者が見つかったという報告[18]もあり，著者も在任末期に同様な経験を持っている。

Relapsing Never：1960年までに発病患者数30～150人の規模の流行が5回あったが，現在は全く発生届出がない。1923年にガーナで発生した流行は患者数150人程度であったが，これは前々年に Guinia に始り，Mali，Upper Volta を経て Accra に侵入し，さらに北緯12度附近の西アフリカ一帯を東進した一連の Pandemie の一部である。

Enteric Fever：日本における腸チフスなどを一括したものだが，この4年間の平均発生数は毎年3100名程度であり，死亡者は平均111名（致命率3.6%）である。1971年以降は若干増えた。首都アクラ市内でさえも本症は伝染性疾患の上位を占めている[19]。原因菌としては *S. typhi* が最も多いが，各種抗生剤に対する耐性菌が増えつつあった[20]。

Food Poisoning：全てが感染症とは言えないが，食中毒が毎年360～560名ぐらい届けられている。この食中毒は人口比で日本より少いが，彼らの衛生環境から考えて，納得ゆかない点である。

Tuberculosis：届出によれば結核患者の発生は絶対数，発生率，死亡率ともに減少の傾向にある。4年間の平均罹患率は10万人中62人で致命率は1.9%であった。ただ，この国の結核の検査は塗抹鏡検が殆んど全てであるから，この数字の背後にある潜在患者の全国的実態は把握できていない。Koch[21]のX-線検査の成績によれば，対象の1.28%が明らかな結核であり多い所では住民の4%が罹患していた村もあった。Sharma[22]の成績では14～44歳の青壮年層に多いことが示されており，ケニアにおける村上[23]の報告同様重症の者が多かった。ガーナにおける患者の発生は1～3月の乾期に多いが，7～9月に多数の死亡者が出るのが特徴である。Ross[24]によれば Rhodesia 西南部の一地域ではアフリカ人の罹患率は10万人当り108人で，ガーナよりも高いが，同地域の白人は10人と明らかに少い。

Anthrax：1970年に130人の患者が発生したが，他の年は10～40名程度であった。しかし，死亡率は16.2%と高い，村落のガーナ人は山羊やめん羊を飼育しており，この家畜の群に Anthrax が流行し，その肉を食べた村民に Gastrointestinal anthrax の流行を起した例[25]がある。

Leprosy：毎年800～1000人（平均922名）の患者が発生している。ガーナ全土に約7万人の患者がいると考えられるが，効果的な治療を受けているのは3割程度と見られる。Chaudhury[26]によれば患者の85%は類結核型であった。1970年現在，ガーナにはライ療養所が7カ所（ベッド数計314）あるが，患者数の多い北部地方にはまだ少い。

Diphtheria：1969年に多数発生したが，20名前後が毎年発生しているに過ぎない。人口比にして，日本と同程度の発生率であるが，致命率はかなり高い。

Pertussis：毎年患者発生数は増加しており，平均発生数は麻疹，水痘に次いで多い。ただし，死亡者数は減少し続けているので致命率は低下して来た。患者発生は4～9月の雨期に集中する傾向があった。ガーナでは百日咳ワクチン（三種混合ワクチン）の接種は1967年で2万人程度で，あまり

大規模には行なわれていないようである。Giel[27]によれば Ethiopia でも小児科新患の4%が本疾患であった。図1に患者発生曲線を示した。

Tetanus：年間1300名以上の患者，200～300程度の死亡者（致命率20%）を出しており，ガーナとしては最も死亡者数の多い感染症の一つである。本疾患の発生について特徴的なことは4年間を通じて殆ど同数の患者が発生しており，年間の各期を通じても平均して患者が現われていたことである。致命率も17%から23%の間で，これまた平均している。発生率は日本よりも高いが，致命率は低いようである。1969年6月から1年間に，アクラの Korle Bu 病院，伝染病棟に入院した84名の破傷風患者のうち40%が死亡しており，15～24歳の者では47%が死亡したと Pobee[28]が報告している。女性の破傷風患者の中には医師以外の手になる人為的流産の際，*Cl. tetani* に感染した場合が多く，きわめて重症となる例が頻繁に見られたと Ampofo[29]が報告している。また Nkuruma[30]によれば新生児の破傷風も多く，3年間に161例の患児が Korle Bu 病院小児科に入院し，64.6%が死亡した。Pobee が主張するように，早急に破傷風に対するワクチン接種を行って，本症を少くする方策が必要であろう。

Poliomyelitis：1970年に前年より若干減少したが，その後，再び増加のきざしが見られた。4年間で総計530名の患者，13名の死者（致命率2.5%）が届出られている。ガーナだけでなくアフリカ諸国においては，近年ポリオが徐々に増加しつつあることが指摘されている[31-33]。いずれにしても，ガーナにおいては *poliovirus* を始め，各種の enterovirus が常時，小児たちの間を循環していることが確認された[34-37]。また，一方では水系感染を疑わせるような事実[15]も認められることから，今後，注意を要する疾患と思われる。

Chickenpox：日本などにおいては，水痘は子供の伝染病と考えられていたが，ガーナでは成人も多数発症している。1969年には約1万人であった患者が翌年は2倍になり，その翌年は6倍以上に増加した。その流行は明らかに季節性が認められ，1～3月の間に患者が多発した。次の麻疹についで患者発生数の多い疾病である。

Measles：1969年を100とすると1971年や72年は300近い値の患者数が届出られている。この数字は感染症のうちでは最も多く，平均して年間67000名以上，死亡者数も破傷風に次いで多い（ただし致命率は低下しつつある）。麻疹も前の水痘と同様に著明な季節性が認められるが，流行のピークは水痘のそれよりも遅く，4～6月であった。麻疹は1～2歳の幼児の死因の中では肺炎，栄養障害，下痢症に次いで多いことが示されている[38]。1967年から始められた天然痘および麻疹撲滅計画[39]が全国的に進められ，1971年の中ばまでに約90万人の子供が弱毒生ワクチンの接種を受けた。この数字はガーナの0～4歳の小児の約6割に相当する。前に述べたように患者発生数は増加しているが致命率が低下したことはワクチンの効果があったものと考えられている。

Bwibo[40]によれば，Uganda ではガーナと異り麻疹の発生に季節性が認められなかったという。1968年までの5年間，やはり増加の傾向にあり，年齢的に発生の最も多いのは1～2歳であった。入院した麻疹患児の死亡率は11.5%であった。藤沢[41]がケニアの Embu で調べた成績では，小児科の入院患者の21.5%が麻疹で，その2割が死亡したと報告している。また，Nigeria の Igbo-Ora では死亡した子供の約23%が麻疹で，大部分1～2歳の年齢層であった[42]。Lagos でも麻疹患児の50%が1.5歳以下であった[43]。

Infective Hepatitis：1969年の届出数に比して，1972年には2.3倍の患者が報告されている。年間平均発生数は8200名，死亡者数が150余で致命率は1.9%である。増加曲線は緩徐だが，着実に増えてきたことは注目された。ガーナ人の血清中のオーストラリア（Au）抗原の検索を行った南らの成績[44-46]によれば健康者の同抗原陽性率は6%であるが，肝炎，黄疸などと診断された患者群では34%が陽性者であった。また，ガーナの流行性肝炎患者の59%に Au 抗原が検出されたという報告[47]もある。一方では，肝炎または黄疸の患者血清のうち16～29%にレプトスピラに対する抗体が見っかったという報告[48]もあるので，届出患者の中には非ウイルス性の肝炎もある程度は含まれているものと考えなければならないだろう。

Rabies：1969年に61名の患者が発生したが，他はいずれも20～30名程度である。しかし，どの時期をとっても0となることはなく数例ずつ散発的に発生していた。死亡率は当然高く，1969年を除き，いずれも100%であった。

Nigeria にも狂犬病は広く蔓延しており[49]，1965年～1968年の4年間に174例の家畜の狂犬病が確認された。

この狂犬病と黄熱病および発生の無かった天然痘を除いて，ウイルス性疾患が最近の4年間にいずれも増加する傾向が見られたのは興味がある。この現象が，真にウイルス性疾患が増加した結果なのか，あるいは単に届出数が増えただけなのかは不明であるが，実際に増加したのであれば，今後，ウイルス性疾患対策は十分に考慮されねばならないであろう。また，ウイルス病が関係者の認識するところとなって，届出が増加したのならば，その原因の一つとして，わが国の行ったウイルス学部門に対する医療協力の影響をあげ得るのではないかと考えている。いずれにしても今後の推移を見守る必要がある。図1に各四半期の麻疹，水痘，肝炎患者発生グラフを示した。

Trypanosomiasis：現在は年に100～150名程度の発生があるのみで，地域も北部地方に限局されているが，これは環境整備による媒介動物の駆除が成功しつつある証拠であると見られる。本症は1933年頃までは殆んど発生が無かったが，1934年から1954年にかけて大規模な流行があり，全国で9万人余の患者が発生した。この流行は1912年 Congo 地方に始り，Cameroon (1920)，Nigeria (1925)，Upper Volta (1830)，Ghana (1934)，Guinea (1939) と続いた一連の大流行の一部である。前出の Relapsing Fever の流行と類似しているが進行方向は正反対である[12]。

Infectious Yaws：この4年間，増加の一途をたどった。1956～1966年に，本症に対する大規模な撲滅計画が全国的に繰り広げられ[50]，計画終了時には最も患者の多かった地域でも0.2%，大部分の地域は0に近くなったが，数年を経ずして再び勢いを盛り返して来たということは熱帯病の絶滅の困難さを物語るものであろう。患者数の増加は図1に示した通りである。

Cerebrospinal Meningitis (C.S.M.)：本症の流行は季節性，地域性が著明に認められる。発生は3月に最も多く，北，中部の4地方で全患者の80%を占める[12]。この時期の北部地方は非常な高温と乾燥した日が続き，サハラ沙漠から飛来する微砂塵風，いわゆるハマターンの季節である。1901～1960年の間にガーナでは C.S.M. の大流行は5回あったが，1906～08年の流行では3万人以上が死亡したと云われる。原因菌としては髄膜炎菌だけではなく，Haddock[51]の調査では Korle Bu 病院の患者では肺炎菌が多かった。季節性は図1にも良く表れている。

Ohpthalmia Neonatrum：毎年700名前後の患者が届出られるが，特に増加の傾向も認められない。死亡者は殆んど無い。

Purperal Pyrexia：毎年600名程度の発生が報告されている。前項と共に出産に関する衛生環境の改善や保健衛生教育の徹底によって発生を抑制できるのではないかと思われる。

その他の疾患は政府発表の統計にはないが，重要な感染症のいくつかについてまとめてみたい。

Malaria：ガーナにおける国民の保健衛生上，マラリアは最も大きな問題の一つである。

1967年の確定死亡者のうち，感染症は約30%であるが，そのうちの四分の一はマラリアであった。すなわち，死亡者1000名のうちの80名はマラリアによって死亡したということである。小児においてはこの比率はさらに高く，実に120人がマラリアで死亡したことになる。おそらく小児科外来患者の半分位はマラリアであろうと推定されている。これらの90%以上が *P. falciparum* による感染であり，*P. malariae* が5%以上，*P. ovale* が1%以上あった。また前2者による混合感染(4～16%)も見られた。これらの比率は岩本[52]のコンゴにおける成績と類似しているが，エチオピアにおける多田[53]のデータとは異なる。Ofusu-Amah[38]が述べているが，1922年に Dr. Marry Magill は5～18歳の子供約500名を調査した結果，25%にマラリア原虫を見つけたと云われる。現在でもこの状態は同様であり，マラリアは栄養障害や肺炎と並んで小児の死因の最たるものの一つである[54]。しかもマラリアは他の疾病と重なって

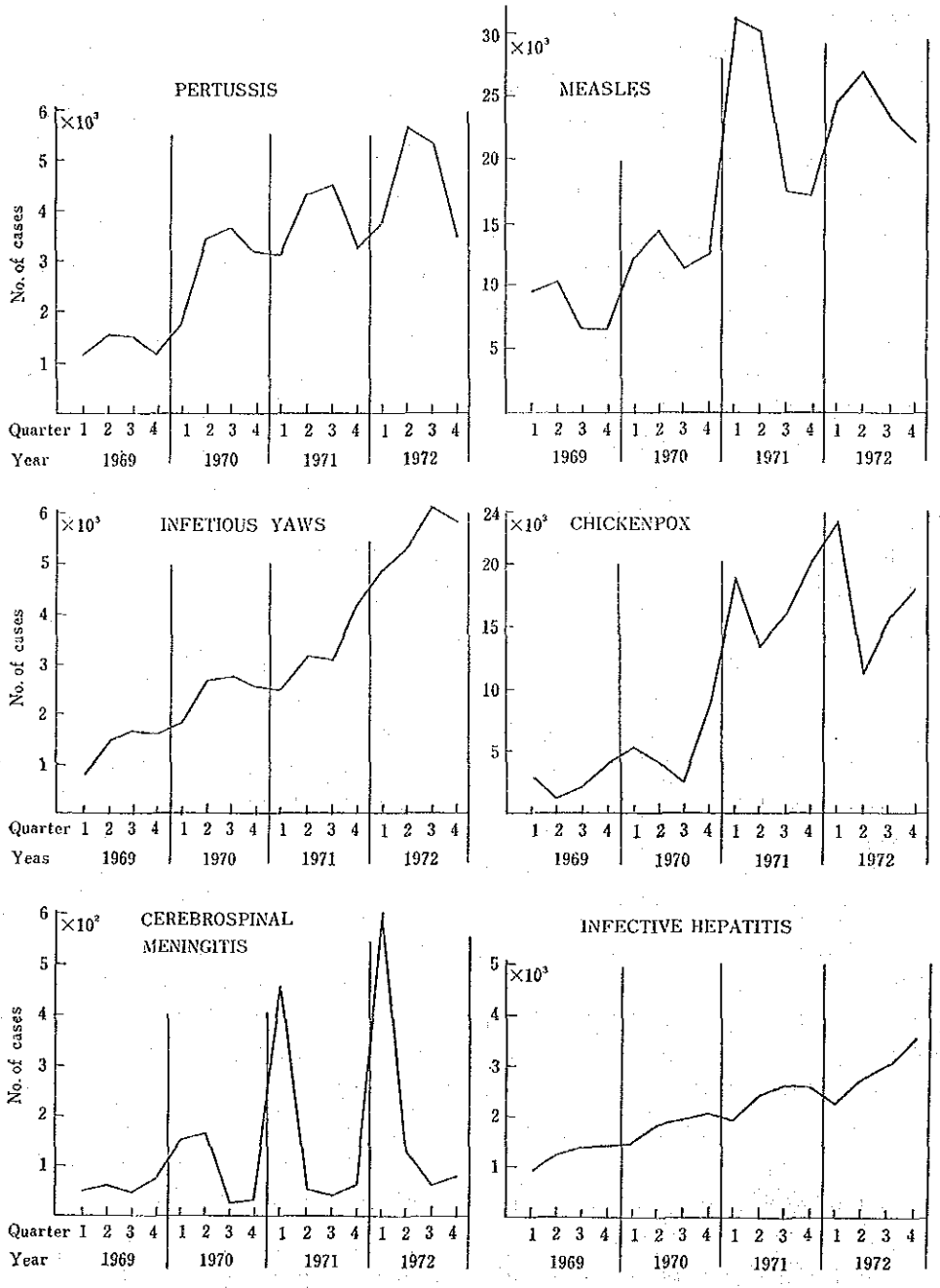

Fig. 1 PERTUSSIS, INFECTIOUS YAWS, CEREBROSPINAL MENINGITIS, MEASLES, CHICKENPOX, and INFECTIVE HEPATITIS in GHANA, 1969-1972. Reported Cases, by Quarter. Quarter 1 ; January-March, 2 ; April-June, 3 ; July-September, 4 ; October-December.

症状を悪化することが多いので事態は深刻である。1967年にいくつかのマラリア撲滅対策が試みられたが，Eastern 地方で行った蚊駆除試験では防虫剤散布を8回行っても十分な効果はあげられなかった。一方，Western 地方のアルミニウム工場で行った試みでは附近の防虫剤散布による蚊の

駆除は不成功であったが，職員に抗マラリア剤を予防投与することによつて，マラリアによる病欠を0に近くすることが出来た。また WHO が中心になって行った Volta 湖附近の町のマラリア絶滅計画は基本的な保健サービスの末端機構の整備が先決であるという理由によって中絶されたままになっている。以上の3つの事業の経験は抗マラリア剤の予防投与と住民に密着した下部保健組織の改善がマラリア撲滅のための必須条件であることを教えている。

Oncocerciasis： 本症は北部ガーナ，特に Volta 河流域に住む住民の間に広く蔓延している。場所によっては住民の5～8%が感染し，そのうちの1～2%が失明していると見られる。ガーナは WHO の協力の下に近隣諸国と共同で本症の撲滅計画を進めている。

Bilharziasis： *Schistosoma haematobium* が最も普遍的に見られるが，稀に *S. mansoni* も検出される。この点は石崎ら[55]の記載と異る点である。本症は極めて局地的に分布する傾向が強く，所によっては15000人の少年のうちの17%が罹患していることが判った。近年 Volta ダムの建設によって中間宿主の巻貝の棲息地域が広がっているので危険は増大しつつあるといわれる。

Shigellosis： ガーナにおいても赤痢は多数発生しており，一般にあまり重視されていないような印象を受ける。1967年の伝染性疾患による確定死亡者のうちの5.1%に当る166名が本症によって死亡しており，腸チフスよりも多い。従って，患者数は相当な数に上っていたものと推定される。1965年から1968年までの4年間に分離された赤痢菌の血清型を調べた Afoakwa[56]の成績によれば82%が *Shigella flexneri* であり，*Shigella dysenteriae* が11%であった。この比率は18年前の1950年頃と殆んど同じ傾向であり，他の西アフリカの国のそれとも類似していた。また，アメーバ赤痢も散見された。

次に，最近，西アフリカで認められた一つの新しいウイルス病について触れたい。

Acute Haemorrhagic Conjunctivitis(A.H.C.) ピコルナウイルスの一種である AHC ウイルス[57]によるこの疾病は世界的な規模で流行し，わが国でも流行した急性の出血性結膜炎である。本疾患は1969年ガーナにおいて最初に流行発生が記録された[58]。当時，アポロ11号の打ち上げがあったのでアポロ結膜炎または Apollo 11 (eye) disease と呼ばれた。南ら[59]の調査によれば本症が流行する前の1968年頃はガーナ人で AHC ウイルスに対する抗体を保有していたものは極めて少数であったが，流行後の1970年になると非常に高い頻度で抗体保有者が見付かった。この疾患がなぜガーナで流行を起すに至ったかは明らかでないが，それまで，病原体が侵入した形跡の認められなかった所に，新たな感染症が発生し，一般住民の間に大流行を起した例といえよう。

Lassa Fever： AHCと対照的なのがLassa Feverである。1969年にナイジェリアの Lassa 地方で伝道に従事していた米国人看護婦が急性の熱性疾患によって死亡し，それを看護した同僚や検査を行った研究者なども次々に感染して死亡したり，非常な重症を呈した[60]。本症は arena ウイルスの一種 *lassavirus* の感染によって起る[61]。現地のナイジェリア人の間で流行することもあるが[62]，一般に，現地住民は血清抗体を保有する者が相当多いにもかかわらず，重症になる例は少いと言われる[63]。古くから，その地方に常在していたウイルスが，たまたまその地に入って来た外国人に感染し，その患者が死亡したために初めて認識されるようになった[64]ものであろう。

ガーナの感染症対策

これらの感染症に対するガーナ政府の防衛組織を見ると何よりも目に付くのは Man power の不足であった。ガーナの保健組織が地域の保健所などを最前線にして組み立てられているのは他と同様である。1971年現在 Health Center が53(建設中：8)，Health Post が37(建設中：67)である。1970年現在，地域中央病院が9，地区病院は34が設置されていた。この他，伝染病病院：4，ライ病院：7，公立病院：12，教会や鉱山の病院が45，私立病院は18であった。これらは前述の地域医務官によって統括されている。感染症の発生届出は各施設の担当者より医務官を通じて厚生省の Biostatistical Unit の Epidemiological Division に

通報される。一部の検査材料は厚生省の Reference Laboratory に送られ，病因が確認されるが，ウイルス検査は全てわれわれが担当した。一般に，患者発生の届出は保健所が最も速く，地区病院，地域中央病院の順だが，診断の正確さでは中央病院が最高で，地区病院，保健所の順となっていた。

なお，感染症ではないがガーナの小児の死因の中で，大きな比重を持っている栄養障害は麻疹や結核，マラリアなどの感染発症と密接な関係があることは指摘されているが[65,66]，この点は本稿の主旨を若干逸脱するので別の機会に譲りたいと思う。

以上，ガーナの伝染病の最近の趨勢について述べた。本稿の主な資料は政府が公式に発表したものを基にしている点，確認された情報ではあるが実情からは若干遊離しており，隔靴掻痒の感があることは否定できない。発生の届出は常に多数の犠牲者が出てから初めて報告されるのが普通である。さらに病原確認はもっと遅れるのが実情であろう。しかも奥地では旧来の習慣に頼ったり，部落の呪術師による治療を受ける場合などが多く[67]，その実態を正確に把握することは極めて困難である。従って，政府発表の数字のみを信用するということは大きな危険を伴うかもしれない。しかし，他に頼り得る統計のない現実において，敢えてそこから出発し，正確な実情把握に取り組んで行かなければならないと考えている。この方面の研究においてなんらかの参考となれば幸いである。

おわりに

ガーナにおいては，現在も，多くの感染症が流行している。その中で特に重要と考えられるものは麻疹，肝炎，破傷風および届出の対象には入っていないがマラリアなどであろう。また，結核，腸チフス，発生数の非常に多い百日咳や水痘，死亡率の高い黄熱病なども切実である。開発途上国の伝染病撲滅の為には予防ワクチンの接種などの直接的な対策と共に，末端保健サービス機構の整備，住民の保健衛生意識向上の教育が並行して進められる必要があろう。

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ORIGINAL ARTICLES:

1. [I-20] CLINICAL ELECTRORETINOGRAPHY (ERG) IN GHANA

By

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Introduction

Numerous investigations have been carried out and many papers have reported on electroretinography (ERG) in various parts of the world. We have used clinical electroretinography to examine over 300 cases at the Eye Clinic, Korle-Bu Teaching Hospital, Accra, from June 1973 to April, 1974. This was carried out by means of an ERG-scope (Handaya, Japan) as part of studies under the Ghana/Japanese Medical Co-operation Programme. We would like to introduce and present the clinical ERG by means of the ERG-scope in this paper.

The ERG, as the evoked series of responses by stimulation of the retina for a brief moment with an intense photo-flash, is an effective, objective examination of retinal function and a valuable aid to diagnosis and prognosis in ocular diseases. A normal ERG shows a negative deflection (a-wave) which is the first response in the ERG. Recently it has been considered that the a-wave is not the first response and that an early receptor potential is found as the fastest response in the ERG. In our present customary methods, however, it can be considered that the a-wave is the first response.

The a-wave is followed by a higher positive deflection (b-wave) which is dependent on the state of dark or light adaptation. The positive wave contains a photopic component (x-wave or bp-wave) and scotopic component (b-wave or bs-wave). Two or three small oscillatory potentials (wavelets) superimposed on the b-wave are found. The b-wave is followed by a slow positive wave (c-wave).

It is important for the clinican to study the a-wave, b-wave and the wavelets. Until recently, the ERG was a complicated and inconvenient laboratory method of investigation. But this ERG-scope with polaroid camera can be operated easily and conveniently for clinical examination.

Materials and Methods

Equipment:

1. Photo-stimulator (electronic stroboscopic flash lamp).
2. Oscilloscope (amplifying).
3. Recorder (polaroid camera).
4. Electrodes (for cornea, forehead and earlobe).
5. Keratin Paste.
6. Shielding Sheet.
7. Copper wire (for earthing equipment).

Fig. 1 shows the ERG-scope and shielding sheet. Fig. 2 shows the connection of the ERG system.

Standards:

1. Sensitivity: 100μV/10mm.
2. Sweep time: 10m sec/10mm.
3. Time Constant: 0.3 sec.
4. Calibration: 20mm for 100μV/10mm.
5. Photo Stimulator: a xenon tube, 5 joule

*Present Address: Dept. of Ophthalmology, Fukushima Medical College. Director Professor: Mutsuo Kajiura.

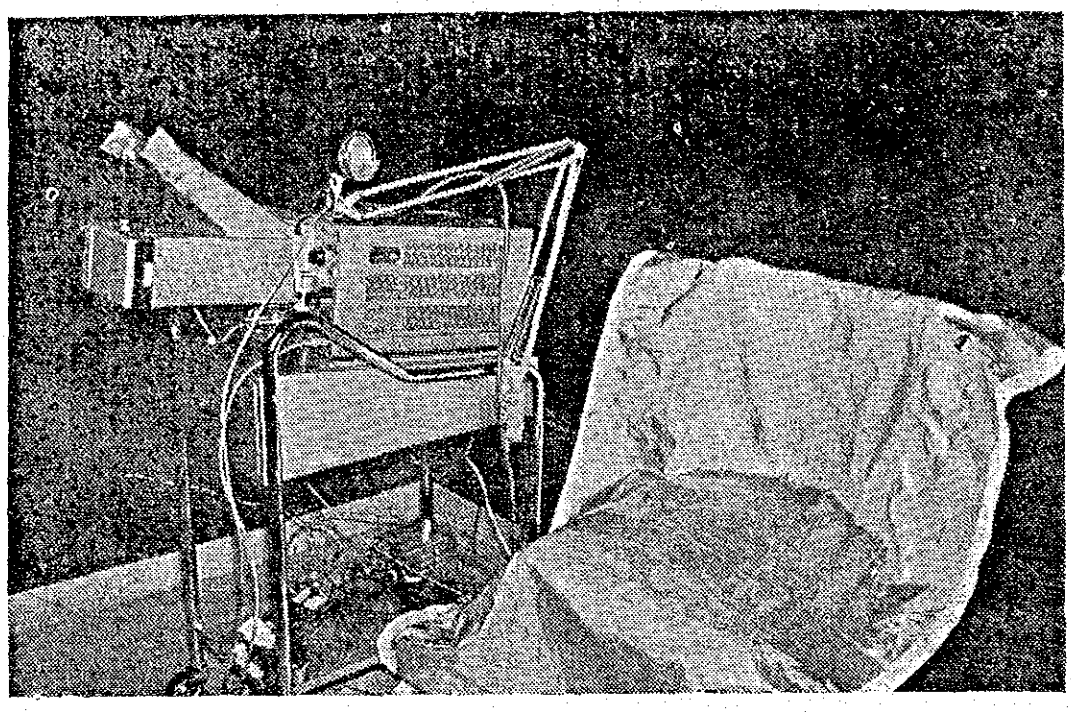

FIG. 1: *Equipment of ERG-scope. Portable instrument on trolley. Shielded sheet on right side.*

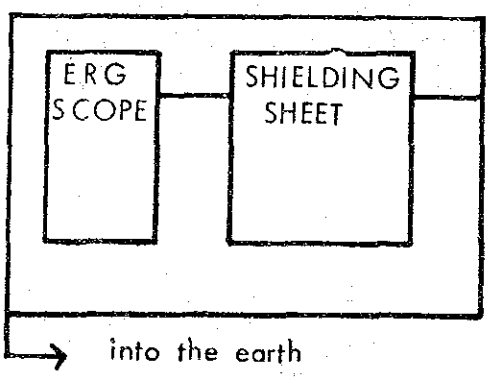

FIG. 2: *Connection diagram of ERG system.*

(low) or 20 joule (high).
6. Time Lag: 20msec (for base line).

Preparation of Patient and Laboratory:

1. Dilatation of patient's pupil using Mydrin-P.
2. The patient is seated on a chair placed on the shielding sheet after shoes or sandals have been removed.
3. Topical anaesthetic is instilled into the patient's eyes.
4. The patient's forehead and earlobes are cleansed and the electrodes inserted or applied.
5. The photo stimulator is positioned 15–20cm in front of the face.
6. The patient is instructed not only to leave both eyes open but also to keep looking straight ahead throughout the test.

Fig. 3 shows a patient under ERG examination. In this case the contact lens electrode of the NOYORI type is in the right eye and the YAMADA type electrode is in the left eye. The electrodes are in contact with the globe of the eye, and there is a forehead band (indifferent Contact lead) on the middle of the forehead, and a contact ground lead is applied to the earlobes.

Light and Dark Adaptation:

In the usual method the photo stimulator is used for light adaptation. In our cases the flash of the fundus camera (Retinapan) was used, i.e., most of the ERGs were taken immediately after fluorescein fundus angiography.

Dark adapted ERGs were recorded after dark adaptation for 5, 10, 15 and 30 minutes.

FIG. 3: *Patient with contact lens electrodes.*

Results

1. The value of each wave in normal cases.

We decided the normal ranges as a result of measuring the ERG of 27 normal cases examined and diagnosed by ourselves.

Photopic ERG shows as below:

Wave	Latency	Amplitude
a	8.18m sec.	254μV
x	30.27 „	330 „
b	37.68 „	328 „

Frequency of appearance of normal pattern of each wave:

a-wave	54 eyeballs/54 (100%)
x-wave	51 eyeballs/54 (94.4%)
b-wave	48 eyeballs/54 (88.9%)

Ratio of amplitude:

x/a	1.30	b/a	1.28

Ratio of amplitude of minimum to maximum in each wave:

a	1.11	x	1.14	b	1.11

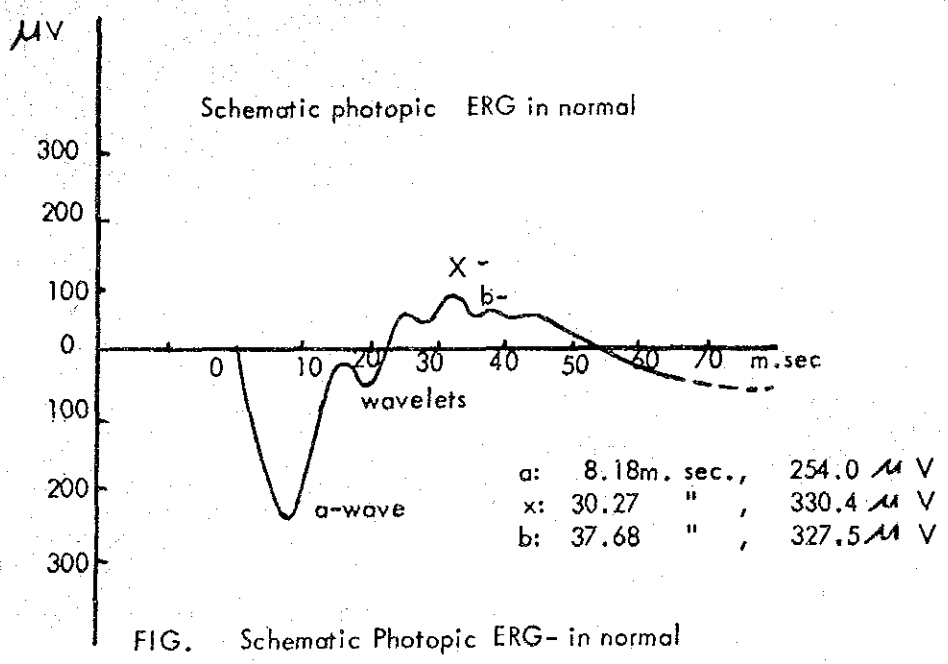

FIG. 4: *Schematic normal photopic ERG.*

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Fig. 4 shows the schematic normal photopic ERG.

Scotopic ERG:

Wave	Latency	Amplitude
a	7.96m sec.	378μV
x	29.51m ,,	480 ,,
b	36.52 ,,	500 ,,

Frequency of appearance of normal pattern of each wave:

a-wave	50 eyeballs/50	(100%)
X-wave	49 eyeballs/50	(98%)
b-wave	49 eyeballs/50	(98%)

Ratio of amplitude:

x/a	1.31	b/a	1.37

Ratio of amplitude of minimum to maximum in each wave:

a	1.15	X	1.10	b	1.12

Fig. 5 shows the schematic normal scoptic ERG. Comparison between photopic ERG and scoptic

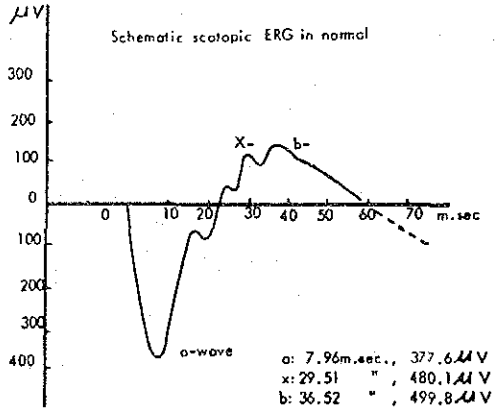

FIG. 5: *Schematic normal scotopic ERG.*

Differences of latency and amplitude:

	Latency	Amplitude
a	8.18— 7.96 = 0.22m sec.	377.6—254.0 = 123.6μV 148% higher
x	30.27—29.51 = 0.66m sec.	480.1—330.4 = 149.7μV 145% higher
b	37.68—36.52 = 1.16m sec.	499.5— 327.5 = 172.3μV 152% higher

2. Practical photopic and scotopic ERG in normal case.

Fig 6 shows photopic ERG of 23-year-old woman. Fig. 7 shows scotopic ERG of the same case as Fig. 6.

The initial spike is the stimulus artifact.

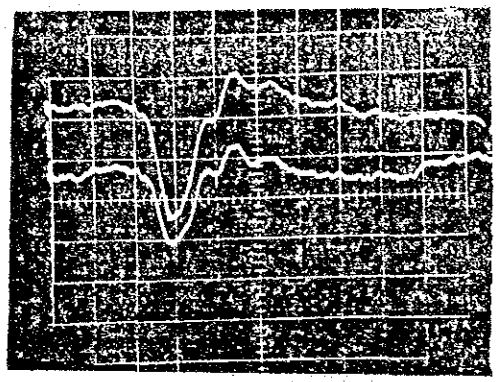

FIG. 6: *Recorded normal photopic ERG.*

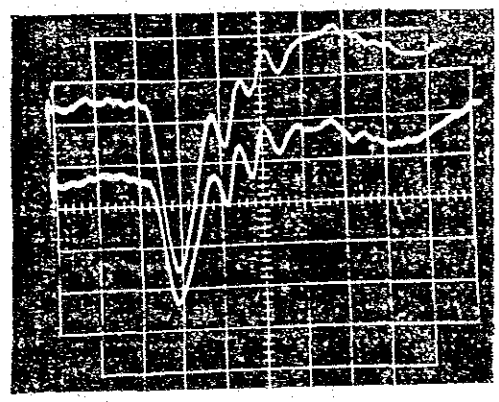

FIG. 7: *Recorded normal scotopic ERG.*

The value of each wave in the right eye is shown as follows:

	Latency (s/o)	Amplitude (s/p)
a-wave	9/9 msec	400/250 μV
x-wave	30/30 ,,	520/320 ,,
b-wave	40/40 ,,	540/290 ,,

These reveal the normal changes in the amplitude of the recovering ERG after dark adaptation (Figs. 6 and 7) compared with little or no change in the amplitude of retinitis pigmentosa (Fig. 11).

3. Fitting of contact lens electrode to Ghanaian eye.

We used two different types of contact lens electrodes (Fig. 8). One used from May, 1973 to August, 1973 is NOYORI's type and the other (right) used from September, 1973 is YAMADA's type originated by one of the authors. Diameter and curvature of the latter are 21 mm and 8.9 mm respectively. Both electrodes show the same value of each wave and the pattern of ERG obtained was the same.

Comparison of the quality of ERG effected by NOYORI'S and YAMADA's:

	Unsatisfactory	Satisfactory	Unsatisfactory %
NOYORI's type ...	70	180	28
YAMADA's type ..	48	882	5.2

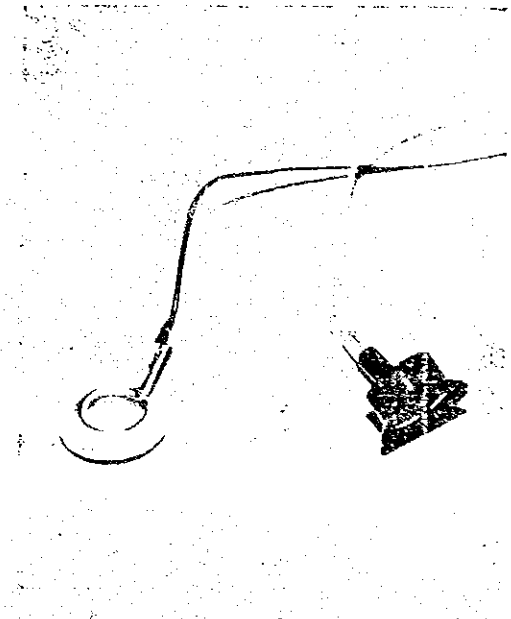

FIG. 8: *Contact Lens Electrodes: Yamada s type (left), Noyori s type (right).*

Fig. 9 shows the type of ERG obtained with both electrodes. The upper one is YAMADA's type, the lower one is NOYORI's type. Fig. 10 shows the ERG affected by poor-fitting (Loose-fitting) of contact lens.

4. Clinical patterns of ERG.

1) The ERG in heredodegeneration of the retina. Retinitis pigmentosa: non-recordable in both eyes (Fig. 11). Sibling of above case: subnormal in right eye (Fig. 12). Nyctalopia of 4-year-old: non-recordable in both eyes

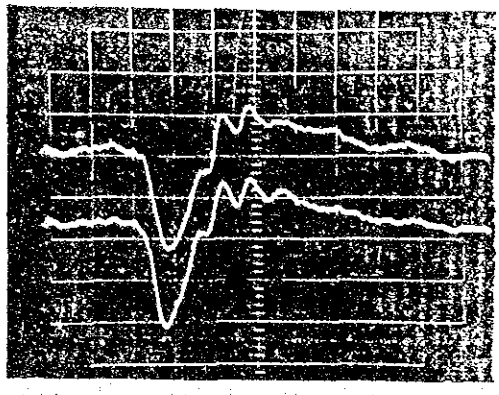

FIG. 9: *ERG using both types of contact lens electrodes: upper is from Yamada electrode, lower is from Noyori electrode.*

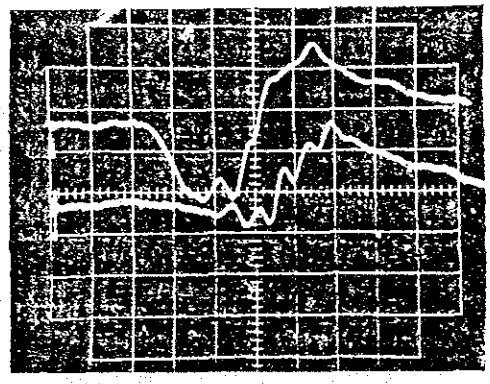

FIG. 10: *ERG affected by unsuitable contact lens electrode.*

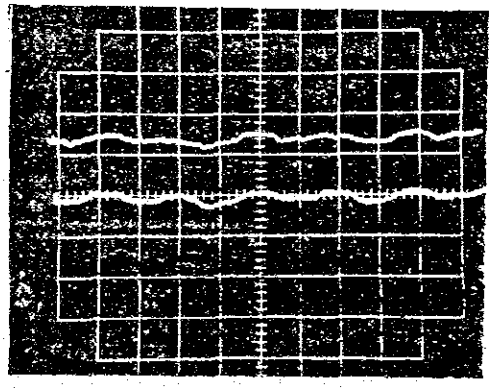

FIG. 11: *ERG in pigmentary degeneration of retina.*

(Fig. 13). Scoptic ERG of the same case (Fig. 14). Oguchi's disease (suspected) of female 14-year-old: reduced ERG (Fig. 15).

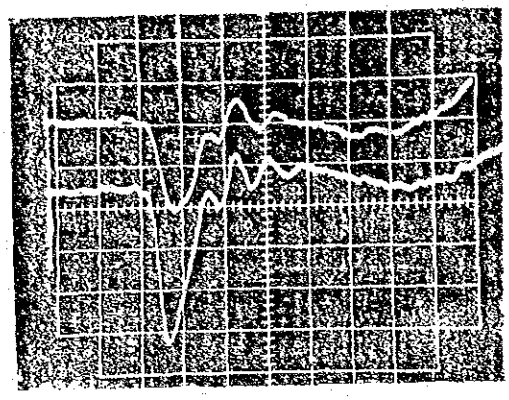

FIG. 12: *ERG of Sibling of Fig.* 11.

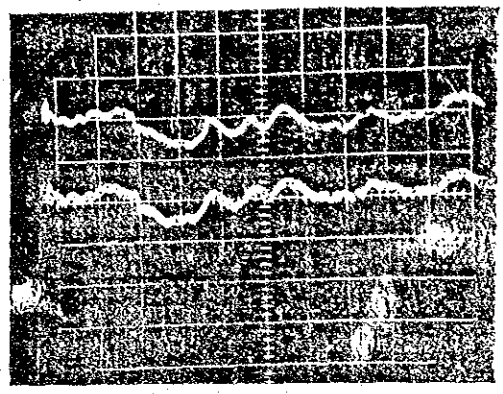

FIG. 15: *ERG in Oguchi's disease* (30 *min. of dark-adaptation*).

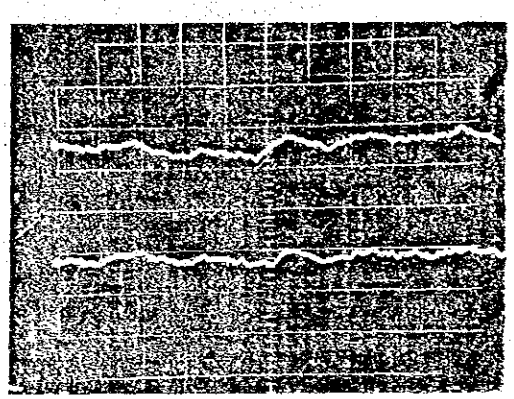

FIG. 13: *Photopic ERG in congenital nyctalopia.*

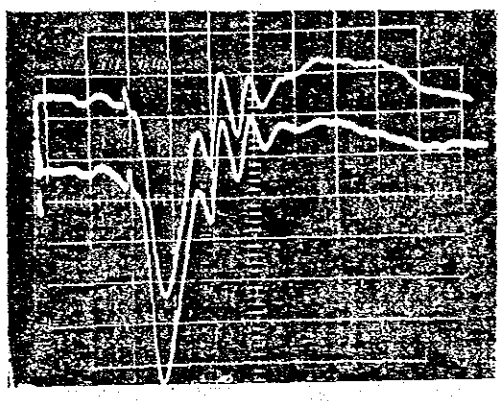

FIG. 16: *ERG of Sibling of case in Fig.* 15.

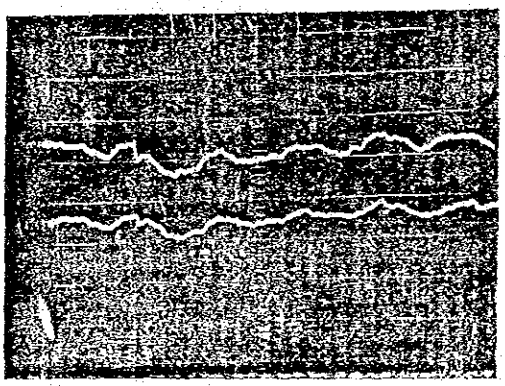

FIG. 14: *Scotopic ERG in same case as Fig.* 13.

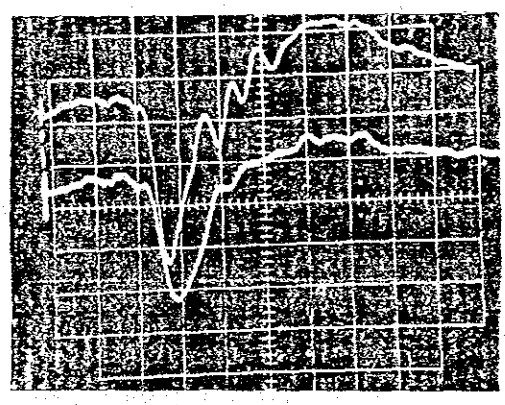

FIG. 17: *ERG in occlusion of central retinal vein occlusion.*

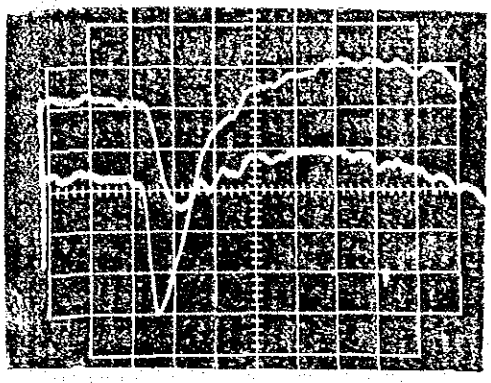

FIG. 18: *ERG in vitreous haemorrhage from sickle cell Hb-c disease.*

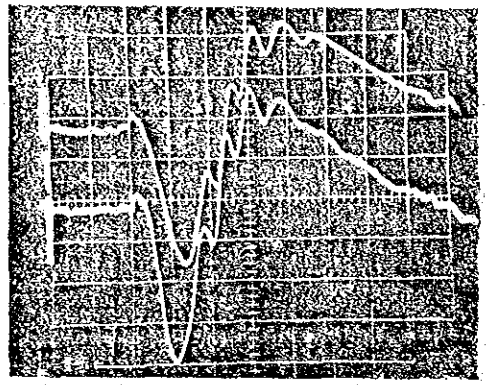

FIG. 21: *ERG in optic atrophy.*

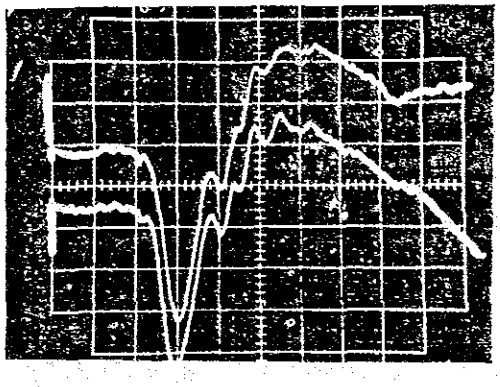

FIG. 19: *ERG in diabetes with hypertension.*

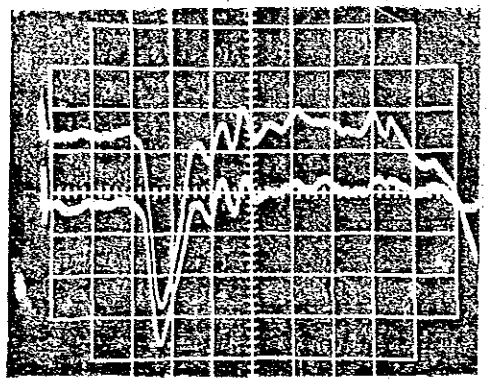

FIG. 22: *ERG in coloboma of optic disc.*

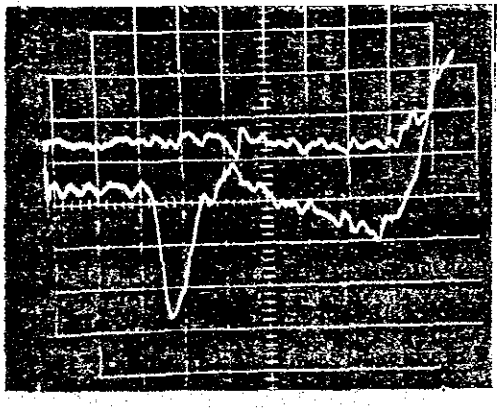

FIG. 20: *ERG in Retinal Detachment.*

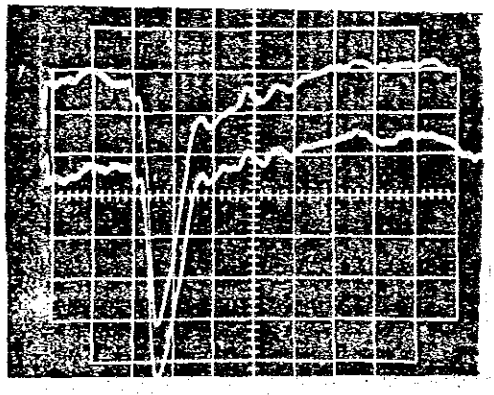

FIG. 23: *ERG in glavicoma.*

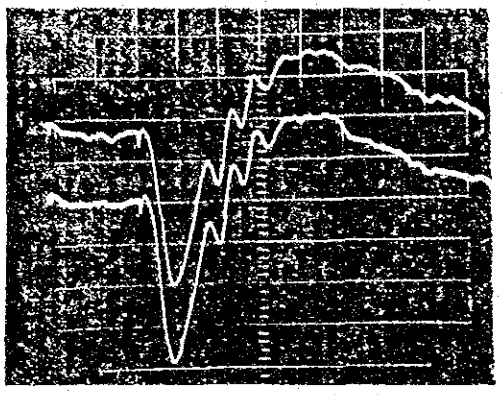

FIG. 24: *ERG central serous charoidopathy*

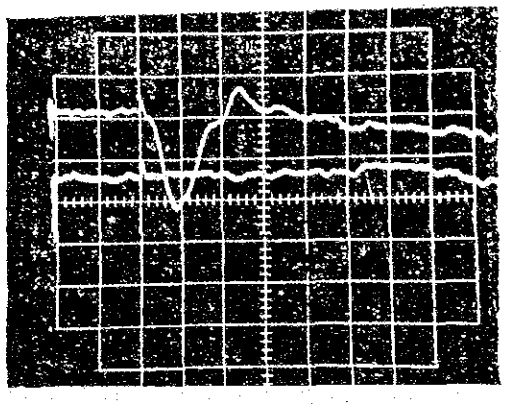

FIG. 27: *ERG in intraocular neoplasm.*

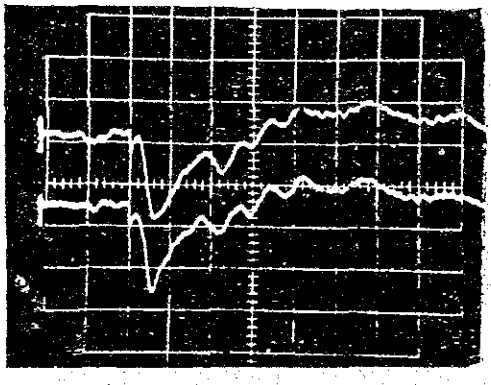

FIG. 25: *ERG in late onchocercal retinopathy.*

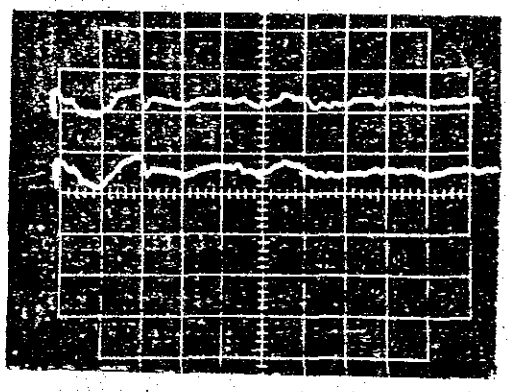

FIG. 28: *ERG in a case of Hodgkin's Disease.*

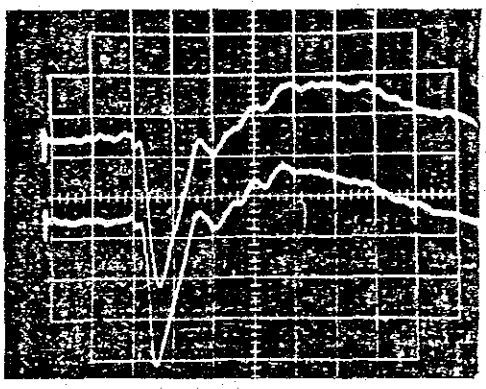

FIG. 26: *ERG in Cataract.*

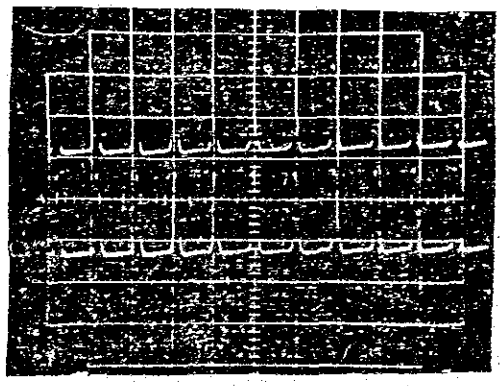

FIG. 29: *Calibration of ERG.*

Sibling of above case of female 10-year-old: normal in both eyes (Fig. 16).

2) The ERG in vascular disturbances.

Left Central Retinal Vein thrombosis: subnormal in left (Fig. 17). Vitreous haemorrhage due to sickle cell disease: reduced wavelets in right eye (Fig. 18). Diabetic retinopathy with hypertension: normal in both eyes (Fig. 19).

3) The ERG in retinal detachment.

Total retinal detachment: non-recordable in right eye (Fig. 20).

4) The ERG in optic nerve disturbances. Optic atrophy: supernormal in both eyes (Fig. 21). Coloboma of right optic disc: normal in both eyes (Fig. 22).

5) The ERG in glaucoma. Open-angle glaucoma: reduced b-wave in both eyes (Fig. 23).

6) The ERG in macular disease. Central serous choroidopathy in right: normal in both eyes (Fig. 24).

7) The ERG in Onchocerciasis. Onchocercal retinopathy: reduced in both (Fig. 25).

8) The ERG in other diseases:

Senile cataract: normal in both eyes (Fig. 26). Intra-ocular neoplasm in left: non-recordable in left (Fig. 27). Hodgkin's disease with optic atrophy: non-recordable in both eyes (Fig. 28).

Discussion

For the sake of simplicity, various factors influencing the ERG are themselves a complex function, i.e., the stimulus, the level of dark or light adaptation, pupil size, electrodes, and even the b u l k i n e s s of the equipment.

For the clinician in ophthalmology, it is desirable to obtain a simplified and accurate ERG for use. The ERG-scope satisfies such demands to some extent. Fortunately, we can use and analyse ERG through many brilliant investigations and reports. We can adopt the term of each response as a-, b-, x-wave and wavelets. Einthoven and Jolly, in 1908, first designated each response (i.e., a-, b-, and c-wave). Granit, in 1933, postulated the existence of three processes called PI, PII, and PIII in his work with cat retina. Riggs summarized Granit's conception in 1956.

In our ERGs, there are some showing a double a-wave. This double a-wave (a-photopic and a-scotopic elements) was demonstrated first by Armington *et al*, in 1952, and they also showed the same elements (b-photopic and b-scotopic elements) in the b-wave. At this time, it was impossible to show in detail whether the apparent doubling was due to the negative phenomena or whether it might be due to superimposed positive elements on the wave. Now, it is well established that it is possible to demonstrate at least two components to both waves. The earlier deflection in either direction is a photopic response, while the later is a scotopic response. Johnson, in 1958, suggested a change of the term x-wave first used by Motokawa and Mita, when he postulated the term a_p and a_s and b_p and b_s for each response. Now term a_1 and a_2 instead of a_p and a_s are usually used. The term x-wave is used in this paper. The exact site of origin of the responses is not known with certainty. It is considered that the a-wave originates in the outer segments of the rods and cones and that probably it is associated with the visual cells, and the b-wave originates in the inner segments of rods and cones and bipolar cells may also play a part. The pigment epithelium is probably responsible for the c-wave. Wavelets are also generated in the inner nuclear layer. It was considered that ganglion cells do not contribute. Recently, with the use of advanced micro-electrodes, it would seem that small conponents can be shown in the ERG originating from the ganglion cells.

Schubert and Bornschein, in 1952, showed that the photopic component is present in night blindness, and that the scotopic component is absent in these individuals. We also present that scotopic component is absent in retinitis pigmentosa, nyctalopia and Oguchi's disease (Figs. 11,13, 14, 15). These are congenital hereditary diseases. The selective depression of the scotopic components of the ERG have been most evident in these diseases. The same phenomenon occurs in retinal detachment (Fig. 20), onchocercal retinopathy (Fig. 2), intra-ocular neoplasm (Fig. 27) and Hodgkin's disease (Fig. 28). These are acquired ocular diseases caused by extensive retinal changes. In onchocerciasis, this may be the first attempted ERG, therefore we would present "The ERG in Onchocerciasis" in a subsequent paper. Oguchi's disease, nearly 100 cases in the world literature, is a rare hereditary disease. Of those cases, 70 or more Japanese

cases and about 30 non-Japanese cases have been reported. Fortunately, Yamada, one of the authors, reported the ERG and Fundus fluorescein angiography of a Japanese case in Japan in 1969. Both (Ghanaian and Japanese cases) ERGs show predominantly negative response and no change after dark adaptation.

Occlusion of the retinal circulation damages bipolar cells and diminishes the amplitude of the b-wave and wavelets. The same depression occurs in severe vitreous haemorrhages. Yonemura *et al.*, in 1962, demonstrated specific changes in the fast oscillatory potentials of the ERG of diabetes. The ERG in diabetes may be normal or subnormal in the advanced stage. Therefore until 1962, ERG was not considered to be of much clinical help. In optic nerve disturbances, the ERG is of normal pattern and sometimes of supernormal shape (Fig. 21). It is a fact that in localized retinal lesions and macular diseases the ERGs are normal (Fig. 24). In retinal detachment, the ERG may give important information regarding both the prognosis for surgical operation and for function of the replaced area. Cataract is often complicated with ocular diseases. The ERG in cataract can be of special value in determining the function of the retina and helps in operative decision.

Most of the artifacts due to motion of eye ball or lid occur at random intervals. They bear no relationship to the stimulus. This can be generally avoided by proper orientation of the patient to the test. The electrode must have the following special features: comfort to the patient, minimum obstruction to the stimulus light and undistorted electrical transmission. Riggs, in 1941, first reported the c.i. electrode; since that many types have been produced. Noyori's type as attached to the ERG-scope is not suitable to the Ghanaian. This causes 28% failure rate of ERG (Fig. 10). Yamada's type, sclerocorneal contact lens, is more suitable to the Ghanaian. It is necessary that more improved contact lens electrodes be produced for Ghanaian or African eyes through studies of their eye globe structure and corneal curvature.

Summary

This paper discusses the introduction of the ERG-scope in clinical practice in Ghana. It records the normal values of ERG of the Ghanaian and shows the pattern of ERG found in a number of cases in clinical ophthalmic practice in Ghana. It shows probably for the first time the ERG in onchocerciasis. A suitable contact lens electrode has been devised to suit the Ghanaian eye.

Acknowledgement

We wish to express our thanks to the staff of the Eye Clinic of Korle Bu Teaching Hospital, Accra, for much assistance; to Mr. Satoru Takano for technical support; to Miss Mercy Brown-Orleans and Miss Emelia Addo of our Ophthalmic Research Laboratory; and to O.T.C.A., Japan, and the University of Ghana Medical School for special support of our programme.

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