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(JICA)

P R E F A C E

The Japan International Cooperation Agency (JICA) has been extending medical cooperation to the University of Ghana Medical School since 1968 as part of the technical cooperation programme of the Government of Japan.

Following the Projects on "Virology and Electron-Microscopy", "Malnutrition and Infectious Diseases" and "Patho-Physiology and Immunology", the Forth Project on "Research and Control of Diarrhoeal Diseases and Improvement of Nutrition" is at present being carried out in Noguchi Memorial Institute for Medical Research.

In commemoration of the past twelve year Japan-Ghana Medical Cooperation, it has been decided to compile the scientific papers earlier published into a book.

I hope that this book would be useful for the further progress of our medical cooperation in the future and at the same time serve to strengthen the friendly relations between our two countries.

I avail myself of this opportunity to express my deep appreciation to the authors and the editors for their kind cooperation to make this publication possible.

M. Hasegawa

Masao HASEGAWA
Executive Director

Japan International Cooperation Agency

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PART I

Scientic Papers Published

「日本医事新報」別刷（第二四八一号）
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ガーナにおけるオーストラリヤ抗原の疫学

[I-1]

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MINAMI, K. and YOKOTA, T.:
AN EPIDEMIOLOGICAL ASPECT OF AUSTRALIA ANTIGEN IN GHANA.
JAPAN MEDICAL J. 2481, 43 - 46, 1971.

During the period between March 1970 and February 1971, 4789 serum samples were collected from Ghanaian healthy persons, blood donors and hospitalized patients with various clinical diagnosis. These sera were tested for the presense of Australia antigen (Au Ag) by means of immunoelectrosyneresis (IES).

Incidence of Au Ag was 34% in the patients with jaundice, 6.2% in healthy parsons, and 5.1% in blood donors. The incidence increased gradually as the age developed, reached to nearly 10% at the age group of 10 - 14 years, then lowered to the order of 3% at the age group of 45 years and older. The incidence in males was higher than in females. In the coastal area a higher incidence was found than either in central forest and northern savanna areas. No significant difference was observed among different tribes.

Basing upon those observations, some possible methods of transmission of Au Ag and its natural history in the tropical Ghana were discussed.

MINAMI, K. & YOKOTA, T.: Epidemiological aspect of Australia antigen in Ghana. Japan Medical J. 2481, 43- 46, 1971.

ガーナにおけるオーストラリヤ抗原の疫学

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はじめに

オーストラリヤ抗原(Au 抗原)は、はじめ Blumberg ら[1]によつてヒト血清中の iso-precipitin の系統的研究の途上、オーストラリヤ原住民の一人にみつけられたものであるが、やがてウイルス性肝炎、特に血清肝炎との関連において注目されるようになつた[2,3]。これと相前後して独立に発見された Prince[4] の Serum-hepatitis-related antigen (SH 抗原)、Krugman ら[5]の人体実験によつて確実に血清肝炎をおこす因子を含む MS-2 と同一であることが明らかにされ[6,7]、Au 抗原は血清肝炎ウイルスそれ自身、または少なくともそれと密接に関連した因子として認識されつつある[8-10]。わが国でも大河内ら[11,12]の輸血および輸血肝炎に関する研究、石田ら[13]の粒子に関する研究とともに、いくつかのすぐれた総説[14-18]が発表され、Au 抗原は最近とみに注目されるに至つた。

われわれは、わが国の海外技術協力事業の一環(註)としてガーナ大学医学部微生物学教室にウイルス学研究室を創設し、一九七〇年はじめより、各種ウイルス病の実験室内診断サービスを開始した。ガーナを含めて西アフリカ地域においては、黄熱病の血清学的診断はきわめて重要なウイルス検査の一つである。一九七〇年を通じて、この目的のためガーナ全土から送られた症例は二六九件に達したが、黄熱病は流行期、流行局地をのぞいては検出されず、わずか九例が診断されたにすぎなかつた。これらの黄熱病の血清検査を要した症例の臨床診断は流行性肝炎、黄疸または黄熱病であり、全体を包含する症状としては黄疸が与えられる。黄熱病と診断できなかつた残る二六〇件の病因に対する手がかりを求めて、これらの血清中の Au 抗原をしらべたところ、その約三四%に Au 抗原が検出された[19]。これを契機としてガーナにおける Au 抗原の全般的分布状況、疾患との関係を知ることが急務となり、血液銀行供血者、入院および外来患者その他の血清をできるだけ多く広範に集め、Au 抗原と Au 抗体のスクリーニングを行なつた。これらの成績の詳細は別に発表する予定であるが、その概要を報告するのが本稿の目的である。便宜上、すでに発表した[19]上記の二六九件の黄疸患者についての成績も含めて記述する。

(註) わが国の中近東アフリカ計画によるガーナ医科大学との医学協力のため、海外技術協力事業団(OTCA)より専門家として、一九六九年一〇月から一九七一年七月までガーナ大学医学部微生物学教室へ派遣された。本研究は医学協力の活動の一部としてガーナにおいて行なわれたものである。

材料および方法

一九七〇年から一九七一年三月までの間にガーナの各地から集められた黄疸患者二六九名[19]、非黄疸患者一〇九二名、ワクチン効果の検討その他の種々の目的でとられた健康者一六六二名および血液銀行供血者一七六六名、合計四七八九名のガーナ人血清を実験に供した(表)。

標準 Au 抗原および Au 抗原に対する抗体(以下、抗-Au と略す)は Dr. B. S. Blumberg, Institute for Cancer Research, Philadelphia (anti-gen : 66540, antibody : 68571), Dr. A. M. Prince, New York Blood Center, New York (antigen : 70-5623, antibody : #1), および Dr. A. Zuckerman, London School of Hygine and Tropical Medicine, London (antigen : 2716, antibody : 5555) から供与を受けた。これらの標準 Au 抗原および抗-Au を用い、寒天ゲル内沈降反応[20]によつてガーナ人血清の中から Au 抗原 (B-5709, B-032) 及び抗-Au (Co-0614, Co-1313) をえらび出し、これらを日常のスクリーニングに用いた。

Au 抗原および抗-Au の検出には Prince and Burke[20] の immunoelectroosmophoresis を適用した。Ouchterlony の寒天ゲル内沈降反応[21]は標準血清の検定およびいくつかの陽性例の同定にのみ用いた。

実験成績

(一)黄疸症例[19]

流行性肝炎、黄疸症または黄熱病と診断され、黄熱病の血清診断を必要とした二六九件中九一名(三三・八%)に Au 抗原が検出された。うち一四才以下が五三名であり、その中の三一名(五八・五%)が Au 抗原陽性であつた。

(二)非黄疸症例

入院および外来患者のうち、前記黄疸症例をのぞいた一〇九二名中六九名(六・三%)が Au 抗原陽性であつた。前記の黄疸症例以外には Au 抗原の存在と特定の疾患との結びつきを結論する成績は得られなかつた。

(三)健康者

黄熱病、麻疹などのワクチン効果の研究、その他の目的で採取された一六六二名の血清中一〇三件が陽性で六・二%であった。

(四)血液銀行供血者

ガーナにおける血液銀行は、すべて献血でまかなわれ、また男性のみが供血者である。一七六六名中九〇名(五・七%)が Au 抗原陽性であった。

表に示すように、黄疸症例を除いてその他の入院・外来患者、健康者、供血者の間で Au 抗原陽性率にほとんど差がないので、これら全体を集計すると四五二〇件中二六二名(五・八%)が陽性であった。健康者、供血者のみを集計すれば、三四二八名中一九三名(五・六%)が陽性であった。年令分布が比較的ひろくわたっている非黄疸患者(一〇九二名)および健康者(一六六二名)の Au 抗原保有率は、それぞれ六・三%、六・二%で、ほとんどひらきがないので、これらを一つの集団として集計し、これら二七五四名について、以下に示す項目に関して検討した。

Prevalence rate of Australia antigen in clinical cases and healthy persons in Ghana (1970-1971)

Clinical group	Number examined	Number positive	Incidence in %
A. Jaundice Cases	269	91	33.8
B. Other Patients	1,092	69	6.3
C. Healthy Persons	1,662	103	6.2
D. Blood Donors	1,766	90	5.1
B+C+D	4,520	262	5.8
C+D	3,428	193	5.6
B+C	2,754	172	6.2

(五)年令分布

〇～四才までは低く一・六%であったが、年令とともに Au 抗原保有率は上昇し、一〇才頃までに八～一〇%となり、二〇才、三〇才台で多少下がり気味となり、四五才以上の年令で三・一%まで下降した。

(六)性別分布

男性六・七%、女性四・九%で男性の方が高かった。

(七)地理的分布

ガーナを海岸サバンナ、森林、および熱帯サバンナ地帯の三つに大別すれば、Au 抗原保有率は海岸サバンナ地帯で低く五・二%であり、森林地帯および熱帯サバンナ地帯で多少高く、それぞれ七・〇%および七・四%であった。

(八)部族別分布

部族の判定は姓名と居住地から行なったが、特定部族への集積性はみられず、むしろ居住地の要素の方が Au 抗原の保有率に影響しているのがみられた。

(九)抗-Au について

抗-Au の保有率は Au 抗原のそれに比べてきわめて低く、黄疸症群で二例(〇・七%)、非黄疸症群で一・〇%(11/1092)、健康者で〇・二%(3/1662)、血液銀行供血者は一七六六名中皆無であった。すべてをひっくるめて四七八九名中一六名(〇・三四%)に抗-Au を検出し得た。

考察

Au 抗原の世界的分布に関しては、Blumberg ら[2]および Prince[22]の広範な研究があり、欧米では〇・一～一・〇%、アフリカ、中南米、アジア、大洋洲などの熱帯地域では欧米諸国の一〇～一〇〇倍にあたる三～二〇%の高率であることが早くから指摘されていた。わが国でも大河内ら[11]の供血者に関する研究で約一%に Au 抗原が発見されている。ガーナ人の Au 抗原保有率については、前記 Blumberg ら[2]の九・五%(九五人中九名)、Prince[22]の六%(一〇〇人中六名)が健康者からのデータであるが、Shulman ら[23]は、endemic hepatitis case 一一二例(二六九の血清検体をふくむ)の五九%に CF 試験によって Au 抗原を検出している。これらはいずれも一〇〇例前後のデータに基づいた報告であるが、今回のわれわれの成績はガーナ全土からできるだけ広範にサンプリングした四五〇〇件以上の血清についての分析結果である。

これらの成績に基づきガーナにおける Au 抗原の分布状況を概括すれば、(一)全体の陽性率が約六%であり、(二)年令分布は幼児期に少なく(一・六%)、一〇～一五才で一〇%近くに達し、以後徐々に下降し、四五才以上で三%台になる、(三)男性に多く、女性に少ない、(四)海岸サバンナ地帯で低く、中央森林地帯および北部の熱帯サバンナ地帯で高い、(五)部族による差はないようである、(六)疾患との関係については黄疸、肝炎を除いて不明であり、健康者と一般患者(非黄疸患者)との間で陽性率に有意の差が見出せない。

この数字(陽性率)をガーナの総人口八五四万六〇〇〇人(一九七〇年の人口統計)にあてはめると、年令構成と年令別陽性率を考慮しても、大凡五〇万人が Au 抗原を保持している計算となる。一方、ガーナにおける輸血はガーナ最大のガーナ大学医学部付属のコレブ病院において一九七〇年中に約七〇〇〇件あり、ガーナ全土を集計して大凡一万件と推定される。その約五%が Au 抗原陽性として約五〇〇人が Au 抗原陽性の供血者であると計算される。この五〇〇人から輸血、注射等によってその一〇〇〇倍にあたる五〇万人へ Au 抗原を伝播することは数量的に不可能に近い。国内の地理的分布では海岸サバンナ地帯に低く、中央森林地帯およびサハラに近い北部の熱帯サバンナ地帯で多いことは、Au 抗原の伝播に関して風土条件(単に気候ではなく、それによってもたらされる生態学的な条件)が関係することを示唆する。このことは、同じ部族でも住居地によって異なった陽性率を示すこと

によつてさらに裏付けされる。男性が女性にくらべて高い保有率を示すことは、性別による感受性の差よりも生活行動様式における性別の差とみるほうが妥当と思われる。もしそうだとすれば、家屋内または集団内よりも、野外での伝播の可能性を示唆する。すでにのべたように輸血、注射等による人工的な伝播はもはや数量的に説明し得ない。

初期の研究者が指摘したように、吸血昆虫の媒介による伝播の可能性[20][21]がきわめてふかい。チンパンジー、アフリカみどりざる、アフリカくもざる、南米きぬざる等の自然の野外動物がAu抗原の注射によりAu抗原を増殖すること、さらにそれが継代できること[39]はこの問題への糸口のひとつと考えられるが、まだ直接証明の段階までには至つていない。最近、カリフォルニアで西アフリカから輸入されたチンパンジーから飼育者が感染し、ウイルス性肝炎をおこしたとの報告[38]はその実体（病因）の分析がまだ発表されていないが、きわめて興味ある報告である。

五〇万人近くのガーナ人がAu抗原を保持するとなれば熱帯病との間になんらかの関連が予想されるが、黄疸症状、流行性肝炎（臨床的）をのぞいては一〇九二名の入院、外来患者について検討した結果、特定の疾患との結びつきをしめす結論は得られなかつた。Blumberg ら[2]のいう第四のカテゴリーの感染（asymptomatic carrier）と簡単にいつていいかどうか、今後さらに、きめ細かく追求することによつて明らかにされよう。また、carrier state を結果するものが、Blumberg らのいう遺伝的なものであろうか。熱帯地域に多いといわれる carrier state はもつと ecological に考察されなければならないと思う。感染発症の問題は、宿主と病原体との間の相関関係にあることは自明のことであるが、宿主の問題をとつてみても、結果として表明された現象のちがいを直ちに宿主の遺伝的な差と言つていいだろうか。胎生期も含めて、熱帯の自然環境のもとにおける人間の生活史を考える場合、二〇人に一人はAu抗原をもち（これはあくまで今回の方法で検出されたという意味で "at least" ということになる）、年中、蚊のいるところで、生まれる前に母体を通じて、また生まれて数日中に蚊によつて微量のAu抗原が、体内に導入される機会は極めて可能性があるように思う。この微量のAu抗原が宿主の immunological state を欧米諸国人とは全く異なつたものにし、一見遺伝的な違いのように見える可能性は充分あろう。

また一方、ガーナのAu抗原と欧米諸国のそれと——少なくとも、寒天ゲル内沈降反応で同一バンドを呈し、電顕でAu抗原と一致する粒子であることは確認しているが——免疫学的に、また virulence の上で同一であろうかという疑問が生ずる。最近の報告によれば、Au抗原には複数の subtype の存在を強く示唆する成績があるということである[23]。

ガーナでは、流行性肝炎が endemic に多く[35]、一九六九年に約五〇〇〇件が厚生省に届出られ、一九七〇年には約七〇〇〇件に達すると見込まれている[36]。季節的には三～五月（雨期のはじめ）に多く、地域的には中央森林地帯、および北部熱帯サバンナ地帯に多いとされている。今回のAu抗原の地域分布とも一致する。古典的な定義による流行性肝炎と血清肝炎の区別は今やくずれつつあり、ウイルス性肝炎はAu antigen associated hepatitis とそれ以外の肝炎に分けられるべきであろう。ガーナでは、しかし、これまで血清肝炎の存在についての考慮は全く払われていなかつた。これは古典的な意味での血清肝炎の伝播様式に関する通念がそうさせたのであつて、今回のわれわれの成績ではこの種の症例の少なくとも三分の一以上がAu抗原を保持しており、より感度の高い CF を用いての Shulman ら[23]の報告によれば、五九%に検出しており、これまで流行性肝炎と考えられていた症例の半分以上は、Au抗原陽性であると予想される。

これらの観察の上にたつて、さらにAu抗原の自然史に関する著者らの想像が許されるなら、次のようにいうことができよう。

Au抗原は太古からアフリカを含む熱帯地域に、輸血などの人工的媒介なしに、ヒトも含めた大自然の中に存在していた。数百年前頃から欧米諸国とアフリカとの交流によつて、Au抗原は欧米に導入されたが、それを、ひろく community の中に伝播、維持するための何ものか（熱帯にはあるが、温帯、寒帯にはないもの）が欠けていたか、不充分であつたため症例は散発に終わり、最近に至るまで社会的問題となることはなかつた。それが、特に戦後の医療サービスの急激な向上、進歩により輸血、注射等の機会が大幅に増し、この人工的なものが熱帯で自然が果たす役割と同じ役割を果たし、欧米でも community の中で独自に伝播、維持することができるようになつて、社会的問題として「黄色い血」がクローズ・アップされるようになつた……と。

要　約

一九七〇年はじめより一九七一年三月までの間に、ガーナ各地から採取した四七八九件のガーナ人血清についてAu抗原のスクリーニングを免疫電気泳動法によつて行ない、次の成績を得た。

Au抗原陽性率は、黄疸症状群で約三四%、無黄疸群が六・三%、健康者六・二%、血液銀行供血者五・一%であつた。Au抗原保持率は年令とともに上昇し、一〇～一四才で一〇%近くに達し、以後徐々に下降し、四五才以上で三%台に達する。男性に多く、女性に少なかつた。海岸サバンナ地帯で低く、中央森林地帯および北部の熱帯

サバンナ地帯で高かった。部族別による差いは見出せなかった。

抗-Auは，〇・三%に検出されたのみであった。

これらの成績に基づいて，Au 抗原のガーナにおける伝播の問題，さらに Au 抗原の自然史について考察した。

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[I-2] ガーナおよび福島県における急性出血性結膜炎に関する血清疫学的研究

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A Seroepidemiological Study on Acute Haemorrhagic Conjunctivitis in Ghana and Fukushima Prefecture, Japan

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A new type of acute haemorrhagic conjunctivitis with unknown etiology occurred in Ghana in 1969. Serum specimens were collected from 210 Ghanaians, 65 at pre-epidemic and 145 at post-epidemic periods. Neutralizing antibody titers of these sera were determined against AHC virus which was isolated in 1971 as a causative agent of acute haemorrhagic conjunctivitis in Japan.

A significant rise in the antibody titers was found in the sera taken at the post-epidemic period, suggesting strongly that the epidemic in Ghana might be caused by the AHC virus. Age distribution of the antibody showed that this epidemic was highly prevalent in the age groups of 0–10 years.

On the other hand, the same type of neutralizing antibody was also found in sera of Japanese, which had been collected in Fukushima Prefecture during 1965–66, when acute haemorrhagic conjunctivitis of this type had not been recognized yet.

緒　　言

1969 年 6 月，アポロ 11 号が人類最初の月面着陸に成功した丁度その頃，西アフリカのガーナにおいて臨床的，疫学的にみてそれまで類例のなかった急性の出血性結膜炎 (Acute Haemorrhagic Conjunctivitis：以下 AHC と略) が爆発的に流行しはじめた[1]。ガーナ人は誰いうとなくこの異例の眼疾患をアポロ病とよんだ。著者らは，たまたま同年 10 月はじめにガーナにおいて本疾患の病因追求の機会を得たが原因ウィルスの分離は不成功に終った[1]。

その後，疫学的，臨床的に同様の眼疾患が病因不明のままナイジェリア[2,3]，中近東，印度，東南アジア[4] 等の広範な地域にひろがり，わが国にも 1971 年 8 月に上陸し，約半年間にわたって全国的に流行した[5-7]。その折，国立予研の甲野博士ら[8] は病因ウィルスとして，ピコルナウィルス群に属するが新型の可能性の強いウィルス (AHC ウィルスと仮称) を分離した。

本報告は，その一部についてはすでに報告したが[9]，日本の流行から分離されたこの AHC ウィルスと病因不明に終ったガーナのアポロ病の病因との間の関連性を血清疫学的に求めようとするものである。また，わが国で AHC が発生する以前に福島県において採取した日本人血清についても同様に AHC ウィルスに対する中和抗体を測定したが，これらについても併せて報告する。

材料および方法

被検ガーナ人血清は，アポロ病流行前の 1968 年 12 月に採取した 65 件[10]，および流行後の 1970～71 年にかけて主としてオーストラリア抗原の疫学的研究[11] のために採取した 145 件であった。一方，日本人血清は福島県におけるポリオの流行予測調査[12,13] のため採取されたも

ので，1965 年に平地区から得た 100 件，1965 年と 66 年に福島地区から得た 100 件，合計 200 件を用いた。これらの血清は −20°C に凍結保存したが，これまで数次にわたり融解をくりかえした。なお，使用直前に 56°C で 30 分間の再加温を行なった。年令区分は 0～10 才，10～20 才，21～30 才および 31 才以上の 4 区分とした。ガーナ人血清の場合，年令不明のものも総平均の算出には除外することなく利用した。

抗原ウィルスには，国立予研の甲野博士より分与をうけた AHC ウィルス，YC-71-670 株（北海道）をサル腎初代細胞で増殖させたものを使用した。中和試験は当教室で継代している ヒト胎児肺 2 倍体細胞の 1 系 (HEL-10，または HEL-11) を用いて行なった。増殖倍地には 10% 牛血清加イーグルの MEM を，維持培地には 1% 仔牛血清加の同培地を使用した。

中和試験は，100TCD_{50}/0.1ml に規正した抗原ウィルス液と等容量の被検稀釈血清とを混和し，37°C で 2 時間，ついで 4°C で 1 夜反応させたのち，その 0.2ml ずつを各血清稀釈段階につき 2 本ずつの HEL-10，または HEL-11 細胞に接種し，34°C で静置培養を行ない，ウィルス対照が丁度 100 TCD_{50} 値を示した 3, 4 日目に最終判定を行なった。血清稀釈は 4 倍段階稀釈とし，中和抗体価は 50% CPE 阻止を示した最高血清稀釈度をもって示した。

実 験 成 績

表 1 および図 1 に要約したように，ガーナにおけるアポロ病流行前の AHC ウィルスに対する中和抗体保有率は，全体としてひくく 1 : 4 のスクリーニングで 23%，1 : 16，で 6%，1 : 64 ではわずか 2% が陽性であった。年令別にみた場合，11～20 才の年令層で多少高く，1 : 4 で 44%，1 : 16 で 13%，1 : 64 で 6% が陽性であった。

これに対して，流行後のガーナ人の抗体保有率，抗体価はともに高く，特に 0-10 才の年令層で高い抗体価が目だった。この年令層の被検血清 25 例中 18 例 (72%) が 1 : 4 で陽性であり，1 : 64 でも 10 例 (40%) が陽性であった。1 : 64 のスクリーニングでの陽性率は，11～20 才で 14%，21～30 才で 19%，31 才以上で 6% であり，10 才以下の 40% が最高であった。

Table 1 Age distribution of neutralizing antibodies against acute haemorrhagic conjunctivitis (AHC) virus in Ghana and in Fukushima, Japan

Place and date	Age group	Number exam'd	Number positive at		
			1 : 4≤	1:16≤	1:64≤
Ghana December 1968 (pre-epidemic)	0-10	2	0	0	0
	11-20	16	7(43.8)*	2(12.5)	1(6.3)
	21-30	17	2(11.8)	0	0
	31≤	10	2(20.0)	0	0
	Unknown	20	4(20.0)	2(10.0)	1(5.0)
	Total	65	15(23.1)	4(6.2)	2(3.1)
Ghana 1970-1971 (Post-epidemic)	0-10	25	18(72.0)	13(52.0)	10(40.0)
	11-20	35	21(60.0)	11(31.4)	5(14.3)
	21-30	31	21(67.7)	13(41.9)	6(19.4)
	31≤	34	19(55.9)	9(26.5)	2 (5.9)
	Unknown	20	15(75.0)	5(25.0)	1 (5.0)
	Total	145	94(64.8)	51(35.2)	24(16.6)
Fukushima, Japan 1965-1966 (Pre-epidemic)	0-10	48	7(14.6)	2 (4.2)	0
	11-20	50	20(40.0)	8(16.0)	2 (4.0)
	21-30	64	27(42.2)	16(25.0)	1 (1.6)
	31≤	38	25(65.8)	13(34.2)	8(21.1)
	Total	200	79(39.5)	39(19.5)	11 (5.5)

* Figure in brackets indicates percentage

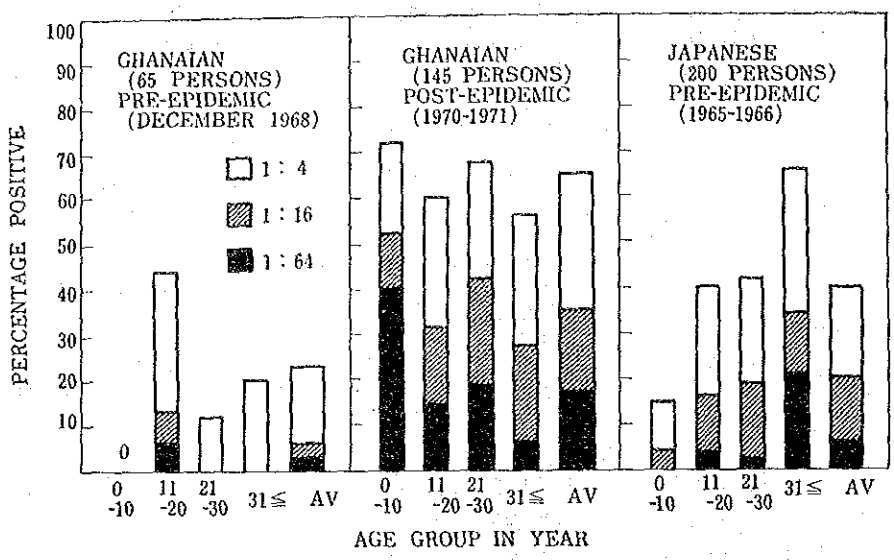

FIG. 1. Age distribution of neutralizing antibodies against AHC virus in Ghana and in Japan (Fukushima prefecture)

一方，福島県の場合は 1：4 のスクリーニングで 0～10 才で 15%，11～20 才で 40%，21～30 才で 42%，31 才以上で 66% が陽性であった。より高値のスクリーニングにおいても抗体保有率は年令とともに上昇し，一般のウィルス感染に見られるようなパターンを示した。

考　　察

ガーナにおいて 1969 年突如として発生した新型の急性出血性結膜炎，現地での通俗名“アポロ病”は，すでにのべたように現地でのウィルス分離が不成功のため病因不明に終った[1]。しかし，病因ウィルスがある流行から分離されなくとも，その集団について流行前および流行後の適当な時期に採取された血清があり，また時間空間を異にしてもそれに類似の他の流行例から病因として分離されたウィルスがあれば，この 2 つの流行の病因の異同については，抗体――特に持続性が長く，特異性の高い中和抗体を測定することによって血清疫学的に推論することが可能である。著者らは，たまたまガーナのアポロ病発生の約半年前の 1968 年 12 月に採取した 65 件，および流行後の 1970～71 年に採取した 145 件のガーナ人血清を日本にもち帰っていた。これらの血清について，日本で 1971 年に発生した同様の疾患から甲野博士らによって分離された AHC ウィルスを抗原として中和抗体を測定した結果，流行前にくらべて流行後は抗体保有率，抗体価共に著明に上昇し，この間にガーナ人集団の間で AHC ウィルスによる広範な感染（流行）のあったことを強く示唆する成績が得られた。この成績は，換言すればガーナのアポロ病と日本の AHC とは同一病因，すくなくとも共通抗原を有する同様のウィルスによるものであったことを示唆している。

一方，わが国においては本疾患は 1971 年 8 月頃にはじめて発生し，それ以前には存在しなかったと考えられている[6,7]。また，今回の観察でも明らかなように，AHC ウィルスはガーナから由来し，パンデミーの一環[5,7]としてわが国に上陸した外来性のウィルスである可能性が強い。しかるに，それから数年前に採取された日本人血清中にすでに本ウィルスに対する中和抗体が証明されたことは，本疾患が臨床的，疫学的にみて全く新しい型の疾患として認識されたことから考えて非常に重要な意味を含んでいる。同様のことがガーナの流行前の血清についてもいえる。

今回測定されたものは，常法に従って解釈すればまぎれもなく AHC ウィルスに対する中和抗体であると考えられる。しかしながら，AHC ウィルスがヒト正常血清中のある種の阻止物質に感受性の強いこと[14]から，今回の測定値（中和抗体価）がこのような阻止物質によって修飾された可能性は否定できない。いずれにせよ，アポロ病または AHC の流行発生前のガーナ人または日本人血清中に AHC ウィルスを不活化する因子が含まれて

いたことは興味あることである。

本疾患の好発年令は成人層であり，小児の症例は極めてすくなかったといわれている[1,7]。しかるに，ガーナ人の流行後の中和抗体保有率，特に 1：64 スクリーニングにおける保有率は 0～10 才の小児で最高であり，この年令層がもっとも多く感染にさらされたことを示している。このように血清学的に観察された感染年令と，臨床的に観察された好発年令との "ずれ" は，本疾患の推定潜伏期が数時間から 24 時間というように極端に短かいことと考え合せて，本疾患の発現におけるアレルギー反応の関与を想起せしめる。

要　　約

1969 年にガーナで発生した新型の急性出血性結膜炎 (AHC：ガーナで俗名アポロ病) は病因不明に終ったが，1971 年同様疾患が日本で流行した際，甲野博士らによって病因として AHC ウィルスが分離された。

著者らはこのウィルスを抗原とし，アポロ病の流行前に採取した 65 件，流行後に採取した 145 件のガーナ人血清について中和抗体価を測定した。その結果，中和抗体保有率はアポロ病の流行をはさんで著明に上昇し，ガーナのアポロ病と日本の AHC とが同一の病因，すなわち AHC ウィルスによって代表されるウィルスによるものであったことを強く示唆する成績が得られた。流行後の抗体価の年令分布は，特に 0～10 才の年令層で高かった。

一方，AHC が日本で流行した数年前の 1965～66 年に福島県で採取した日本人血清 200 件について同様に AHC ウィルスに対する中和抗体価を測定した結果，かなりの割合で中和抗体が証明された。このことは，ガーナの流行前の血清中にもある程度中和抗体が証明されたことと相まって興味ある事実である。

本研究を遂行するにあたり，抗原ウィルスおよび標準抗血清の分与をたまわり，また終始激励をたまわった国立予研の甲野礼作博士に対して深甚の謝意を表す。

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原　著

[I-3] ガーナにおける Australia 抗原の分布状況に関する研究

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緒　言

Australia (Au) 抗原は，はじめ Blumberg[1] によつてヒト血清中の *iso-precipitin* の系統的研究の途上で Australia の原住民からみつけられた．Au 抗原に対する特異的抗 Au 血清をウイルス性肝炎患者の血清と反応させると電子顕微鏡レベルで確認できる凝集反応をおこす18～25mμ の粒子がみつかり[2]，ウイルス性肝炎患者の肝細胞が抗 Au 蛍光抗体で特異的に染色され[3]，さらに，Au 抗原はウイルス性肝炎，特に従来の分類による血清肝炎患者の血中から高頻度に検出されることが確かめられ，Au 抗原がウイルス性肝炎と密接な関係にあることが確実となつた[4)~8)]．

Au 抗原の世界的分布に関しては Blumberg ら[9] および Prince[7] の広範な研究があり，欧米および日本[10]で 0.1～2％，東南アジア，アフリカ，大洋州などでは３～20％の高率であることが指摘された．この中で Blumberg はガーナについて調査し，Au 抗原が 9.5％と高率に検出できることを明らかにした[9]．

一方，ガーナでは臨床的に流行性肝炎と診断される患者が多く，これと Au 抗原が高頻度でガーナ人から検出できることとの結びつきを予想し，調査した結果，流行性肝炎と診断された者から *Immunoelectrosyneresis*(I.E.S.) 法で34％，ＣＦ法で54％の高頻度で Au 抗原が検出された[11]．ガーナのように風土病的に Au 抗原陽性のウイルス性肝炎が存在し，健康者からも Au 抗原が高頻度で検出される地域の Au 抗原の伝播は欧米諸国とちがつていることが推察でき，その基礎資料を得る目的で Au 抗原の年令別，地域別，部属別頻度を求め，比較検討したので報告する．

調査対象地

ガーナをその風土，特に気候と植物帯を主にして海岸サバンナ (Coastal)，熱帯雨林 (Forest)，ギニアサバンナ (Savanna) の３つの地域にわけた．図１は，これら３地域と各地域内の1970年の流行性肝炎発生数[12]と人口比の発生率を示し，さらに各地域内の疫学調査地区とその被検者数を示している．

1．海岸サバンナ地域 (Coastal Area)

ギニア湾沿岸に位置し，1970年の届出流行性肝炎患者数は1256名，人口比の発生率は 0.064％であつた．調査地区は主都の Accra と Tema，Winneba の３地区で年間平均気温は 26.4℃，年間降雨量は 700～ 800㎜，年間平均湿度は80～85％である．

この地域は主として Ga, Fanti, Ewe 部属の居住地である．

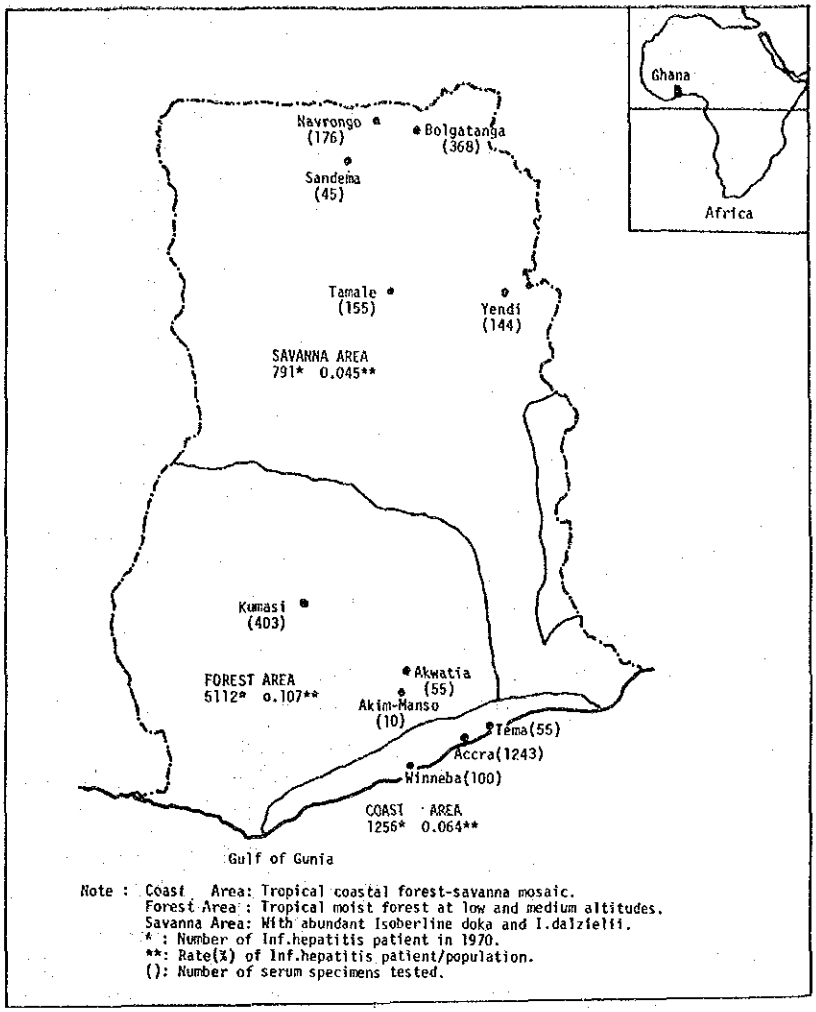
2．熱帯雨林地域 (Forest Area)

ガーナの中央に位置し高温多湿の地で，Kumasi, Akwatia, Akim-Manso の３調査地区の周囲はジャングルである．年間平均気温は 26.0～26.5℃，年間降雨量は Kumasi で1455㎜，他の２地区で2100㎜，年間平均湿度は86～89％である．1970年の届出流行性肝炎患者数は5112名で，人口比の発生率は 0.107％と肝炎の多発地域である．この地域は主として Akan 部属の居住地である．

3．ギニアサバンナ地域 (Savanna Area)

ガーナの北部に位置し高温であるが雨量は少なく草原地である．1970年の届出流行性肝炎患者数は791名で，人口比の発生率は0.045％であつた．調査地区 Tamale, Yendi, Navrongo, Bolgatanga, Sandema の５地区の年間平均気温は 28℃前後，

Fig. 1. Epidemiologically Investigated Area for Australia Antigen in Ghana



年間降雨量は1100㎜，年間平均湿度は55～60％である．主として Tamale は Dagomba, Yendi は Komkomba, Navrongo は Grushie, Bolgatanga は Frafra の各部属の居住地である．

実験材料と方法

1．被検者と被検血清

被検者は上記の各地区の健康者および黄疸症をのぞく病院入院外来患者を対象とした．採血は1970年3月から1971年2月までの間に行なわれ，総数2754件の血清を検査した．被検血清は採血後できるだけすみやかに血清分離を行ない，使用前まで－20℃に保存された．

2．Au 抗原と抗 Au 抗原抗体

Au 抗原の同定および抗 Au 抗原抗体（Au 抗体）の検定用標準 Au 抗原は Blumberg (66540), Prince (70—5623), Zuckerman (2716) よりそれぞれ分与されたものを使用した．実験に主として用いた Au 抗原は，ガーナ人の供血者から陽性の血清（B-5664）を選び使用した．Au 抗原の同定は，ゲル内沈降反応で行ない，電子顕微鏡で18～

25mμ の Au 抗原粒子の存在を確かめた.

標準Au抗体は, Au 抗原同様 Blumberg(68571), Prince (#1), Zukerman (5555) より分与された. Au 抗原検出のスクリーニングに使用した Au 抗体は, 抗原検出率の高い3名のガーナ人血清 (0614, 1317, T-64) を用いた. これら Au 抗体の検定は標準 Au 抗原, Au 抗体とのゲル内沈降反応で行なつた.

3. *Immunoelectrosyneresis* (I.E.S.)

被検血清中の Au 抗原検出のためのスクリーニング試験は I.E.S. を用いた. 方法は基本的には Prince[13] に準拠した. 緩衝液は pH 8.6, イオン強度0.02のベロナール緩衝液で泳動には 0.9%に寒天を加えたものを用いた.

結　果

1. 年令, 性別 Au 抗原頻度

総被検血清2754件を男女別にわけ, さらに0～4, 5～9, 10～19, 20～29, 30～44, 45才以上の各年令区分にわけて Au 抗原頻度を示したのが表1である. 男性全体の Au 抗原頻度は 6.7% (135/2002), 女性全体では 4.9% (37/752) であり, 男性が高く女性が低くかつた. 年令別に比較すると, 両群とも0～4才, 45才以上で低く, 10代が高かつた. 特に0～4才の年令層で低く, 10代までは年令とともに上昇し, 20代以上では年令とともに下降の傾向がみられた.

Table 1. Age Distribution of Australia Antigen in Ghanaians specified by Sex (1970-1971)

Age group	Male			Female		
	Number tested	Number positive	%	Number tested	Number positive	%
0—4	107	3	2.8	84	0	0
5—9	109	11	10.1	43	2	4.7
10—14	277	33	11.9	206	11	5.3
20—29	721	47	6.5	241	18	7.5
30—44	531	32	6.0	109	5	4.6
45 & over	257	9	3.5	69	1	1.4
Total	2002	135	6.7	752	37	4.9

Table 2. Age Distribution of Australia Antigen in Ghanaian Children (1970-1971)

Age group	Number tested	Number positive	Per cent positive
0—2M*	20	0	0
3—6	38	2***	5.3
7—12	25	0	0
1Y**	33	0	0
2	34	0	0
3—4	41	1	2.4
5—6	49	2	4.1
7—9	103	11	10.7
10—14	148	22	9.3

*: Month, **: Year
***: Both of two cases were five months old.

2. 低年令層の Au 抗原頻度

年令別比較により0～4才の低年令層の Au 抗原頻度が他の年令層と比較してかなり低く, 10代までの Au 抗原頻度の上昇率が高いので, ガーナ全体の低年令層をさらに細分化し比較したのが表2である.

0～2カ月児の Au 抗原頻度は0% (0/20), 3～6カ月児で 5.3% (2/38, 2例の陽性はいずれも5カ月児), 7カ月から2才児までは92例中1例も陽性なしで, 2才児までの頻度は 1.3% (2/150) であつた. Au 抗原頻度は3～9才までは年令とともに上昇し, 7～9才で10.7%とピークになり, 10代で高く, 20代以後下降していた. 年令区分5～6才と7～9才間で Au 抗原頻度が 6.5%も上昇していたのがめだつた.

3. 地域別 Au 抗原頻度

表1の成績を3つの地域別にわけ, 年令別 Au 抗原頻度を示したのが表3である.

Coastal Area の総被検血清の Au 抗原頻度は 5.2% (73/1398), Forest Area では 7.1% (33/468), Savanna Area では 7.4%であり, Coastal Area が他の2地域と比較し若干低くかつた. 年令別比較では, 3地域とも10～19才で高く, 45才以上で低くかつた. Coastal Area の0～4才が 0.6% とかなり低かつたのと, Sabanna Area の5～9才が20%と高かつたのがめだつた.

Table 3. Age Distribution of Australia Antigen in Ghanaians specified by Area (1970–1971)

Age group	Number tested	Number positive	Per cent positive
Coastal Area			
0—4	163	1	0.6
5—9	66	2	3.0
10—19	72	5	6.9
20—29	566	33	5.8
30—44	415	27	6.5
45 & over	116	5	4.3
Total	1398	73	5.2
Forest Area			
0—4	10	1	10.0
5—9	41	2	4.9
10—19	115	10	8.7
20—29	141	13	9.2
30—44	90	5	5.6
45 & over	71	2	2.8
Total	468	33	7.1
Savanna Area			
0—4	18	1	5.6
5—9	45	9	20.0
10—19	296	29	9.8
20—29	255	19	7.4
30—44	135	5	3.7
45 & over	139	3	2.2
Total	888	66	7.4

Table 4. Prevalence of Australia Antigen in Ghanaians specified by Tribe (1970–1971)

Tribe	Number tested	Number positive	Per cent positive
Ga	237	12	5.1
Fanti	235	7	3.0
Ewe	120	3	2.5
Akan	597	51	8.5
Frafra	255	19	7.5
Grushie	165	11	6.7
Dagomba	219	15	6.8
Ga-Adangbe	46	3	6.5
Komkomba	23	2	8.7
Other & unknown	857	49	5.7
Total	2754	172	6.2

Table 5. Prevalence of Australia Antigen in Ghanaians specified by Area and Tribe (1970–1971)

Tribe	Number tested	Number positive	Per cent positive
Coastal Area			
Ga	227	12	5.3
Fanti	161	3	1.9
Ewe	90	1	1.1
Akan	170	12	7.1
Dagomba	39	3	7.7
Ga-Adangbe	21	1	4.8
Other & unknown	690	41	5.9
Forest Area			
Ga	10	0	0
Fanti	18	1	5.6
Ewe	10	1	10.0
Akan	313	25	8.0
Dagomba	27	1	3.7
Other & unknown	83	4	4.8
Savanna Area			
Fanti	56	3	5.4
Ewe	20	1	5.0
Akan	114	14	12.3
Frafra	244	18	7.4
Grushie	163	11	6.8
Dagomba	153	9	5.9
Ga-Adangbe	21	1	4.8
Komkomba	22	1	4.5
Other & unknown	95	8	8.4

4. 部属別 Au 抗原頻度

表4はガーナ全体の被検血清の部属別 Au 抗原頻度を示し，表5はそれをさらに地域ごとに細分し比較したものである．Au 抗原頻度がもつとも低くかつたのは Ewe の 2.5% (3/120)，高かつたのは Akan の 8.5% (51/597) と Komkomba の8.7% (2/23) であつた．概して Coastal Area を中心として居住している Ga, Fanti, Ewe の各部属が低く，Foaest, Savanna Area を主に居住している Akan 等の部属が高い傾向を認めた．Fanti, Akan の部属は3地域からある程度の血清を集め比較できたが，Coastal Area に住んでいるグループが他の2つの地域に住んでいるグループより Au 抗原頻度は低くかつた．

討 論

Au 抗原の世界的分布に関しては Blumberg[9],

Prince[7] らの広範な研究があり，その中で Au 抗原が熱帯地域で高率に検出されることが指摘された．ガーナに関しては Blumberg[9] により 9.5% の高率であることが報告され，アフリカではほかにセネガルの成人で9%，ウガンダの成人で2%という報告[8]がある．今回，われわれの調査した黄疸症患者を除くガーナ人の Au 抗原頻度は 6.2%と高率であつた．地域別比較で5～8%の範囲，部属別比較で2～8%の範囲で差が認められた．同一部属でも概して Forest, Savanna Area に住むグループの Au 抗原頻度が高いことから，部属差は地域差によるものと考えられる．地域差を有意とするかどうかはさらに例数を増し検討する必要があろう．熱帯アフリカでなぜ Au 抗原が広い地域に高率に浸淫しているのか，その伝播様式を欧米諸国の成績とあわせ比較することは今後の重要な課題である．われわれは，別報[14]で記したように，輸血や注射らの人工因子によらない熱帯の風土条件の関与を考える．

ガーナ内でも近代化が進んでいるCoastal Area の Au 抗原が概して低くかつたことや，アメリカ在住の黒人間で低いという成績[9]は上記の考えを示唆している．また，Forest Area で Au 抗原頻度が高いこと，アフリカ産のチンパンジー，アフリカくもざるなどの霊長類で Au 抗原が増殖し継代できること[6][15]，アメリカで西アフリカから輸入したチンパンジーから飼育者が感染してウイルス性肝炎に罹患したとの報告[16]等をあわせると，Au 抗原のジャングル内での人と動物間での伝播も推察できる．

年令別に検討すると Au 抗原頻度は，0～2才の幼児期で 1.3%と低く，10代までは年令と共に上昇し，7～14才の年令層で10%前後となり，以後，年令とともに下降していた．われわれはガーナで臨床的に流行性肝炎と診断される患者が多く，その患者から I.E.S. で34%，CF で54%の高率で Au 抗原が検出されることをつきとめた[11]．特に15才以下の年令層の患者からは I.E.S. で59%，CF で72%の高率で Au 抗原が検出された．この15才以下の年令層は，今回の疫学調査で Au 抗原頻度の上昇率の高かつた年令層であつた．Au 抗原頻度の高かつた10代，20代でウイルス性肝炎患者が多く，その年令層のウイルス性肝炎患者から高率に Au 抗原が検出できることは Au 抗原とウイルス性肝炎との結びつきを確かにしている．15才以下で Au 抗原頻度が高い事実の説明として，この年令層の Au 抗体保有率が低いという最近の *Passive hemagglutination* や *Radio immunoassay* を用いての成績[17]-[18]があげられる．

0～2カ月の幼児では20例中1例も Au 抗原陽性者は検出できず，3～6カ月児で38例中2例（陽性の2例はいずれも5カ月児）がみつかり，以後7カ月から2才児までは検出されなかつた．新生児，幼児の Au 抗原頻度が低い要因は，第1に母胎内の Au 抗原の垂直伝播はなく，出生後感染により Au 抗原に汚染されるため，第2に母胎内を含めて早期に Au 抗原に汚染されても量的に検出できないことなどが考えられ，それに Au 抗原の潜伏期が関与してこよう．新生児，4カ月未満の幼児から Au 抗原陽性例が1例も検出できなかつた成績は，感染後に Au 抗原が検出されるまでの潜伏期が1～3カ月である[19]という報告とあわせると Au 抗原の胎内伝播は少ないものと考える．Schweitzer ら[20]は，2例であるが母親が出産後に抗原陽性の肝炎を発病するとしばらくして幼児も Au 抗原陽性に変化し，産道もしくは出生後感染による Au 抗原の伝播を報告している．

Au 抗原頻度が男性に高く女性に低い事実は世界的な傾向である．この要因が遺伝的なものか社会的環境のちがいによるのか今後の解析が必要である．

結　論

1970年8月から1971年2月の間にガーナ全土から2754件の被検血清を集め，I.E.S. 法で Au 抗原の検出を行ない，性別，年令別，地域別，部属別 Au 抗原頻度をもとめ比較検討し次の結果を得た．

1．黄疸症患者を除いたガーナ人の総被検血清2754件の Au 抗原頻度は 6.2%であつた．

2．Au 抗原頻度は年令により差が認められ

た．すなわち0～2才児の低年令層で 1.3%と低く，10代までは年令とともに上昇し10%前後となり，10代，20代で高く，20代以後年令とともに下降し45才以上の高年令層では 3.1%であつた．

3．男性の Au 抗原頻度が 6.7%と女性の 4.9%より高かつた．

4．地域別，部属別検討で Au 抗原頻度は若干の地域差が認められたが，部属差は認められなかつた．

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An Epidemiological Investigation of Australia Antigen in Ghana

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Between March 1970 and February 1971, 2754 serum samples collected from various parts and tribes of Ghana were examined for Australia (Au) antigen by means of immuno-electrosyneresis.

The epidemiological view of Au antigen in Ghana was summarized as follows:

1. Over all incidence of Au antigen in the general population (healthy persons and non-jaundiced patients) of Ghana was 6.2%.
2. Au antigen was rarely found in infants and young children under age 4. The incidence gradually increased, reaching a maximum of about 10% at age 10, then gradually decreased with age.
3. Higher incidence was calculated for male (6.7%) than that of female (4.9%).
4. No obvious difference was observed among various tribes.
5. Lower incidence of Au antigen was noted in the Coastal area (5.2%) than in Forest (7.1%) and Savanna areas (7.4%).

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[I-4] ガーナにおける Australia 抗原とウィルス性肝炎

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Australia Antigen and Viral Hepatitis in Ghana

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In the period between March 1970 and February 1971, 4,789 Ghanaian sera collected from 269 clinical cases with jaundice, 1,092 various clinical cases without jaundice, 1,662 healthy persons and 1,766 healthy blood donors, were examined for Australia (Au) antigen by means of immuno-electrosyneresis (IES), complement fixation (CF) test and electron microscopy.

Incidence of Au antigen in the Ghanaian patients with jaundice was 33.7% by IES and 53.9% by CF test. In the children under 14 years old, the incidence was 58.5% by IES and 71.8% by CF test. Clinical diagnoses given to these patients were infectious hepatitis, jaundice and/or yellow fever.

Incidence of Au antigen was 6.3% in the other (non-jaundice) patients, 6.2% in the healthy persons and 5.1% in the blood donors. Apart from the jaundice patients, no particular relationship was established between any other clinical group and presence of Au antigen. Because the group of non-jaundice patients and that of healthy persons showed almost the same incidence of Au antigen, and also because they, taken together, covered a wide range of age and geographical distributions, the two groups were integrated for further investigation. The epidemiological view of Au antigen in Ghana based on this investigation was as follows:

1. Overall incidence of Au antigen in the general population of Ghana was 6.2%.
2. Au antigen was rarely found in infants and young children under age 4. The incidence increased with age, reached a maximum rate at age 10, and decreased gradually thereafter.
3. Incidence in males (6.7%) was higher than that in females (4.9%).
4. Incidence in the Coastal area (5.2%) was lower than either of those of Forest (7.1%) and Savanna (7.4%) areas.
5. No marked difference was observed among different tribes.

緒　　言

1964 年 Blumberg[1] によって発見された Australia (Au) 抗原は，その後ウィルス性肝炎，特に B 型肝炎との関連において注目をあびるに至った[2-4]。

Au 抗原の世界的分布に関しては Blumberg[4], Prince[5] らの報告があり，ガーナもふくめた熱帯諸国では，日本[6] や欧米諸国に比較して高率に Au 抗原が検出されている。

一方，熱帯アフリカでは肝疾患が多く，ガーナでは 1970 年に流行性肝炎として約 7,000 名が厚生省に届出られた。さらに熱帯における各種肝疾患から Au 抗原が高率に検出され両者の因果関係を強く示唆している[7]。

著者らは，ガーナにおいて黄熱病の血清学的診断を行なってきた。1970 年の間にガーナ各地から 269 症例の検体が送られたがそのほとんどが黄熱病の陰性例であった。そこで著者らは，黄熱病の血清診断で陰性となった症例の病因を求めて Au 抗原の検出を試みた。さらに日

本や欧米諸国と疫学状況を異にするガーナでの Au 抗原の浸淫を調査するため約 4,500 件の血清を集め Au 抗原の検出を試みたので報告する。

実験材料と方法

1. 被検血清

a. 黄疸症患者群： 1970 年 3 月から 12 月の間に黄熱病の血清診断のためガーナ各地から送付された 269 名の患者血清を用いた。これら患者の臨床診断名は表 2 に示すように流行性肝炎，黄疸症，黄熱病等であった。

b. 非黄疸症患者群と健康者群： 図 1 に示すようにガーナ各地から入院患者 1,092 名，一般健康者 1,662 名，献血者 1,766 名を対象とし，血清は 1970 年 3 月から 1971 年 2 月までの間に採取し，その総数は 4,520 件に達した。

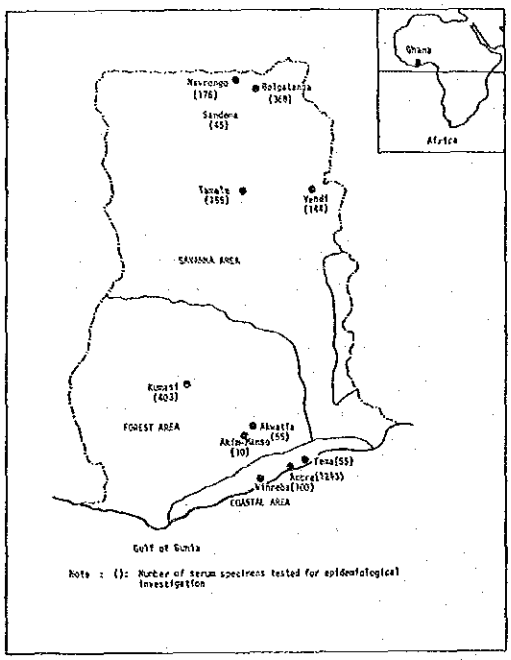

FIG. 1. Investigated area for Australia antigen in Ghana.

2. Au 抗原と Au 抗体

標準 Au 抗原と Au 抗体には，Blumberg より分与されたものを用い，被検血清のスクリーニングにはガーナ人血清中から Au 抗原 (B-5706) および Au 抗体 (614, 1317, T-64) を選び用いた[8-9]。

3. Immunoelectrosyneresis (I.E.S.)[10]

詳細は別報[9] に記した。

4. 補体結合反応 (CF)[10]

黄疸症例についてのみ CF でも Au 抗原の検出を行なった。試験は血清稀釈 1：16 で行ない，抗補体作用の陽性例は 1：64 まで稀釈して行なった。使用した Au 抗体は，4 単位のヒト由来の抗体 (T-64) である。

5. 電子顕微鏡による Au 抗原の検出

Au 抗原の形態学的確認と一部黄疸症についてのみ電顕で Au 抗原の検出を試みた。被検血清 2ml を PBS で 10ml とし，50,000rpm (日立 65 PAT ローター) で 3 時間遠心を 2 回行なった。最後の沈査を 0.2ml の蒸留水に浮遊させ，2% 燐タングステン酸 (pH 7.2) による Negative 染色を行なった。

観察は直接倍率 6~8 万倍で行ない，1 視野最短約 3 分間観察し，陰性の場合は 10 視野計約 30 分の観察にもとづいて結論を出した。

6. アルボウィルスの HI 試験

黄疸症例のみに行なったアルボウィルスの HI 試験は Clarke ら[11] に準じて行なった。試験には Dakar のパスツール研究所の Dr. Bress から分与されたマウス脳内培養による黄熱病ウィルス (FN 株) の Sucrose Acetone 抽出による SA 抗原を用いた。被検血清は Kaolin 処理で非特異的物質の除去を行なった。

実験成績

黄疸症患者群の成績

1. 地域別 Au 抗原陽性率： ガーナを Coastal, Forest, Savanna の 3 地域 (図 1，別報[9] 参照) にわけて Au 抗原陽性率を示したのが表 1 である。総被検例 269 件の Au 抗原陽性率は I.E.S. で 33.7%，CF で 53.9% であった。地域別比較では Coastal Area がひくく，Forest, Savanna Area が高かった。

TABLE 1 Incidence of Australia antigen among patients with jaundice specified by area

Area	Number tested	I.E.S.*		CF**	
		Number positive	%	Number positive	%
Coastal	121	37	30.6	57	47.1
Forest	91	32	35.2	48	52.7
Savanna	57	22	38.6	40	70.2
Total	269	91	33.7	145	53.9

* I.E.S.: Immuno-electrosyneresis.
** CF: Complement fixation test.

TABLE 2　Australia antigen and HI antibodies against group B arbovirus among patients with jaundice classified by clinical diagnosis and signs

Clinical diagnosis and signs	Number tested	Au Antigen				Arbo-B	
		I. E. S.		CF		HI*	
		Number positive	%	Number positive	%	Number positive	%
Infectious hepatitis	188	66	35.1	103	54.8	4**	2.1
Jaundice	37	12	32.4	25	67.6	1	2.7
Jaundice with fever	22	9	40.9	10	45.5	0	0
Yellow fever	22	4	18.2	7	31.8	4	18.2
Total	269	91	33.7	145	53.9	9	3.4

* Hemagglutination inhibition test (Yellow fever SA antigen was used.)
** Number of cases in which a definite (4-fold or more) rise in HI titer between 2 suitably spaced sera was demonstrated.

2. 臨床診断，症状別の Au 抗原陽性率：　表2に示すように流行性肝炎と診断された 188 例については I. E. S. で 35.1%，CF で 54.8% が Au 抗原陽性であった。単に黄疸症と診断された 37 例については I. E. S. で 32.4%，CF で 67.6%，黄疸と発熱がみられた 22 例については I. E. S. で 40.9%，CF で 45.5% がそれぞれ陽性であった。黄熱病と診断された 22 例の Au 抗原陽性率は I. E. S. で 18.2%，CF で 31.8% であった。ペア血清間でアルボウィルス B 群（黄熱病ウィルス）に対し 4 倍以上の HI 抗体価の上昇がみられた例は 9 例 (3.4%) であった。

3. 年令別 Au 抗原陽性率：　黄疸症例の年令別 Au 抗原陽性率は I. E. S. で 0～14 才で 58.5% と極めて高く，15 才以上では 28.9% で両年令層との間に有意差がみとめられた。CF でも同様の傾向がみられ 0～14 才で 71.7%，15 才以上で 51.4% であった。

4. 電子顕微鏡による Au 抗原検出：　I. E. S. と CF の 2 つの方法で確認した Au 抗原陽性 13 件，陰性 37 件の計 50 件の血清を選び電顕で Au 抗原の検出を試みた（表 4）。Au 抗原陽性例からは 100%，陰性例からも 70.3% の高率に Au 抗原様粒子が検出できた。

非黄疸症患者群と健康者群の成績

1. 疾患別 Au 抗原陽性率：　表5に示すように黄疸症を除く各疾患別の Au 抗原陽性率のうち高かったのは成人の Anaemia の 26.7%，Chicken pox の 11.4% 等であった。熱帯病のうち Malaria は 0% (0/17)，西アフリカに多い Burkitt tumor は 2.9% と低く，黒人に多い Sickle cell anaemia には陽性例がなかった。

2. 全体的 Au 抗原陽性率：　総被検血清 4,520 件を非黄疸症患者，健康者，献血者の各群にわけてその Au 抗原陽性率を示したのが表6である。3 者間に有意差が認められなかったので，年令分布，地理的分布が比較的広くわたっている非黄疸症患者群と健康者群を 1 つの集団として集計し，これら 2,754 件について以下に示す

TABLE 3　Age distribution of Australia antigen among patients with jaundice

Age group	Number tested	I. E. S.*		CF**	
		Number positive	%	Number positive	%
0-4	15	8	53.4	8	53.4
5-9	19	12	63.2	15	78.9
10-14	19	11	57.5	15	78.9
Sub-total	53	31	58.9	38	71.8
15-19	36	10	27.7	17	47.2
20-29	81	30	37.0	47	58.0
30 & over	66	13	19.7	30	45.2
Sub-total	183	53	28.9	94	51.4
Unknown	33	7	21.2	13	49.5
Total	269	91	33.7	145	53.9

* I.E.S.: Immuno-electrosyneresis.
** CF : Complement fixation test.

TABLE 4　Detection of Australia antigen in patients with jaundice by electron microscopy.

Au-Antigen*	Number tested	E-microscopy	
		Number positive	%
Negative	37	26	70.3
Positive	13	13	100.0
Total	50	39	78.0

* Confirmed by two methods, I.E.S. and CF.

TABLE 5 Australia antigen among hospitalized patients specified by clinical diagnosis.

Disease	Clinical diagnosis	Number tested	Number positive	%
Infectious disease	Chicken pox	35	4	11.4
	Leprosy	28	1	3.6
	Malaria	17	0	0
	Tuberculosis (pulmonary)	35	1	2.9
Enteric disease	Abdominal pain	37	6	16.2
	Diarrohea	17	0	0
	Other enteric disease	13	0	0
Respiratory disease	Broncho pnemonia	17	1	5.9
	Chest pain	29	2	6.9
	Commaa cold	42	4	9.5
Tumor	Burkitt tumor	34	1	2.9
Surgical disease	Abscess	13	1	7.7
	Injury (fracture)	31	2	6.4
	Swollen	19	3	15.8
	Hernia	11	0	0
Gynaecologicl disease	Abortion	23	0	0
	Delivery	41	0	0
	Pregnancy	11	1	9.1
Other disease	Anaemia (adult)	15	4	26.4
	〃 (children)	10	0	0
	〃 (sickle cell SS)	20	0	0
	〃 (sickle cell SC, SF)	10	0	0

TABLE 6 Prevalence rate of Australia antigen in clinical cases and healthy persons in Ghana.

Clinical group	Number examined	Number positive	Incidence in %
A. Jaundice case	296	91	33.7
B. Other patient	1092	69	6.3
C. Healthy person	1662	103	6.2
D. Blood donor	1766	90	5.1
B+C+D ·····	4520	262	5.8
C+D ·····	3428	193	5.6
B+C ·····	2754	172	6.2

項目について検討した。

3. 性，年令別 Au 抗原陽性率： 表7に示すように男性全体の Au 抗原陽性率は 6.7%，女性全体では 4.9% であり男女差がみられた。年令別に比較すると両群とも 0～4 才でひくく，10 代までは年令とともに上昇し，20 代で高く以後年令とともに下降の傾向がみられた。

4. 低年令層の Au 抗原陽性率（表8）： 低年令層の Au 抗原陽性率の変動が著るしいのでこれをさらに細分し比較した。0～2 ケ月児の Au 抗原陽性率は 0%，3～6 ケ月で 5.3%，7 ケ月から 2 才児までは陽性例なしで 2 才児までの陽性率は 1.3% であった。

3～9 才間では年令とともに上昇し，7～9 才で 10.7% とピークを示した。年令区分 5～9 才間で 6.5% も陽性率が上昇していた。

5. 地域別 Au 抗原陽性率（表9）： Coastal Area 全体の Au 抗原陽性率は 5.2%，Forest Area では 7.1%，Savanna Area では 7.4% であり Coastal Area が他の 2 地域と比較し低かった。年令別比較では 3 地域とも 10 代で高く 45 才以上で低かった。Savanna Area の 5～9 才が 20% と高いのがめだった。

6. 部属別 Au 抗原陽性率（表 10, 11）： Au 抗原陽性率がひくかったのは Ewe の 2.5%，高かったのは Akan の 8.5% と Komkomba の 8.7% であった。概して Coastal Area を中心に居住している Ga, Fanti, Ewe の各部属で低く，Forest, Savanna Area を主に居

TABLE 7 Age distribution of Australia antigen in Ghana specified by sex (1970-1971)

Age group	Male			Female		
	Number tested	Number positive	%	Number tested	Number positive	%
0-4	107	3	2.8	84	0	0
5-9	109	11	10.1	43	2	4.7
10-14	148	17	11.5	89	5	5.6
15-19	129	16	12.4	117	6	5.1
20-29	721	47	6.5	241	18	7.5
30-44	531	32	6.0	109	5	4.6
45 & over	257	9	3.5	69	1	1.4
Total	2002	135	6.7	752	37	4.9

TABLE 8 Age distribution of Australia antigen in Ghanaian infants and children

Age group	Number tested	Number positive	%
0-2 month	20	0	0
3-6	38	2*	5.3
7-12	25	0	0
1 year	33	0	0
2	34	0	0
3-4	41	1	2.4
5-6	49	2	4.1
7-9	103	11	10.7
10-14	237	22	9.3
15-19	240	22	9.2
20-29	962	65	6.8
30-44	640	37	5.8
45 & over	326	10	3.1
Total	2754	172	6.2

* Both of the two were five months old.

TABLE 9 Incidence of Australia antigen in Ghana specified by age and area.

Area	Age group	Number tested	Number positive	%
Coastal Area	0-4	163	1	0.6
	5-9	66	2	3.0
	10-19	72	5	6.9
	20-29	566	33	5.8
	30-44	415	27	6.5
	45 & over	116	5	4.3
	Total	1398	73	5.2
Forest Area	0-4	10	1	10.0
	5-9	41	2	4.9
	10-19	115	10	8.7
	20-29	141	13	9.2
	30-44	90	5	5.6
	45 & over	71	2	2.8
	Total	468	33	7.1
Savanna Area	0-4	18	1	5.6
	5-9	45	9	20.0
	10-19	296	29	9.8
	20-29	255	19	7.4
	30-44	135	5	3.7
	45 & over	139	3	2.2
	Total	888	66	7.4

住している Akan, Frafra, Komkomba の各部属が高い傾向を示した。同一部属を地域別に比較すると Fanti, Ewe の部属は Coastal Area に居住している集団で 1% 台, Forest, Savanna Area に住んでいる集団で 5% 以上と地域差がみられた。一方, Dagomba のように逆の成績を示す部属もみられた。さらに Coastal Area 内の比較で 1% 台の Fanti, Ewe と 7% 台の Akan, Dagomba の部属の共存もみられた。

考　　察

熱帯アフリカは肝疾患が多く，ガーナでも厚生省に届出られた流行性肝炎患者数は 1969 年で 5,000 名，1970 年で 7,000 名にも達した[12]。さらにガーナでは黄疸を主たる臨床症状とする疾患が多く，実験室内診断のないままその診断名は黄疸症，黄熱病，レプトスピラ感染症などとなっている。

これら黄疸症群の病因をつきとめるべく，ガーナ各地から流行性肝炎，黄熱病などと診断された 269 名の患者を対象として Au 抗原の検出と黄熱病ウィルスを用いてのアルボウィルス B 群に対する HI 抗体価を測定した。

TABLE 10 Incidence of Australia antigen in Ghana specified by tribe.

Tribe	Number tested	Number positive	%
Ga	237	12	5.6
Fanti	235	7	3.0
Eew	120	3	2.5
Akan	597	51	8.5
Frafra	255	19	7.5
Grushie	165	11	6.7
Dagomba	219	15	6.8
Ga-Adangbe	46	3	6.5
Kombomba	23	2	8.7
Other & unknown	857	49	5.7
Total	2754	172	6.2

TABLE 11 Incidence of Australia antigen in Ghana specified by area and tribe.

Area	Tribe	Number tested	Number positive	%
Coastal Area	Ga	227	12	5.3
	Fanti	161	3	1.9
	Ewe	90	1	1.1
	Akan	170	12	7.1
	Dagomba	39	3	7.7
	Ga-Adangbe	21	1	4.8
	Other & unknown	690	41	5.9
Forest Area	Ga	10	0	0
	Fanti	18	1	5.6
	Ewe	10	1	10.0
	Akan	313	25	8.0
	Dagomba	27	1	3.7
	Other & nknown	83	4	4.8
Savanna Area	Fanti	56	3	5.4
	Ewe	20	1	5.0
	Akan	114	14	12.3
	Frafra	244	18	7.4
	Grushie	163	11	6.8
	Dagomba	153	9	5.9
	Ga-Adangbe	21	1	4.8
	Komkomba	22	1	4.5
	Other & unknown	95	8	8.4

その結果，アルボウィルスB群に対しペア血清間で4倍以上のHI抗体価の上昇を示したものは9名（3.4%）であった。この9名は1970年3~5月に Akwatia で黄熱病の流行があったときに採取された者であり，散発的に発生した黄疸症例からはこのような例はみられなかった。

黄疸症群の Au 抗原陽性率は I. E. S. で 33.7%，CF で 53.9% であり，一部電顕で検出すると I. E. S.，CF で陰性であった血清中からも 70% の割で Au 抗原様粒子が観察できた。さらに感度の高い IAHA[13]，RI[14] 法を用いることにより検出率はさらに高くなることが推察できることから，ガーナの黄疸症の大部分は Au 抗原陽性の B 型肝炎であるといってよいであろう。

ついで Au 抗原陽性の肝炎が多発する背景を調査する目的でガーナ全土から 4,520 件の血清を集め I. E. S. で Au 抗原の検出を試みた。

今回，著者らが調査した非黄疸症群の Au 抗原陽性率は 5.1~6.3% であり日本[6] や欧米諸国[4-5] に比較しかなり高率であった。この原因として黄疸症以外にも Au 抗原が高率に検出される熱帯特有の疾患の有無を調査した。その結果 20% 以上の陽性率を示したのは成人の Anaemia のみであった。この原因は治療法の輸血によるものであろう。

年令別比較で示した Au 抗原陽性率のカーブは世界的傾向であり[15]，熱帯ではそれが増幅されたかたちであらわれている。最近，PHA，RI 法を用いることにより Au 抗体が高率に検出できるようになった。その成績で Au 抗体保有率は一般のウィルスと同様に年令とともに上昇し成人で 20~40% であることがわかってきた[16]。従って Au 抗原の年令分布は Au 抗体保有率との相関関係で説明できる部分が多い。

新生児，幼児の Au 抗原陽性率が低いという事実も興味あることである。この問題へのアプローチは，母体内での垂直伝播の有無[17]，Au 抗原の潜伏期[18]，さらに母体免疫などを考慮しながら検討される必要がある。

次に Au 抗原陽性率は，地域別比較で 5.2~7.4%，部属別比較で 2.5~8.7% の差がみられた。ガーナでは部属により主な居住地が決っている。従って同一部属で地域別比較ができにくい。一部比較できた Akan，Fanti，Ewe の各部属では Coastal Area に住んでいる集団が他の Forest，Savanna Area に居住している集団より低く，部属差より地理的因子が重要であるように思われた。しかし，同一地域内（Coastal Area）の比較で 1% 台の Fanti，Ewe と 7% 台の Akan とが共存しており一概に部属差を否定できない。地理的，民族的分布が他のウィルス感染症より問題となるのは Au 抗原感染後の生体反応に遺伝子の関与がかなり考えられるからである。Blumderg[4] は，Au 抗原陽性率の高い東南アジアの Cebu，Bougainville の家族調査から Au 抗原の Carrier となる生体要因として常染色体劣性（autosomal recessive）遺伝子が関係しているといっている。一方，

大林[19]は，Blumberg の成績と適合しない高率に Au 抗原（Au 抗体）が検出される肝硬変家系を報告している。

なぜ熱帯で Au 抗原陽性率が高いのか遺伝学的背景を考慮しながらその伝播様式を欧米諸国の成績とあわせ比較することは重要である。著者らは別報[8-9]でも記したように輸血や注射らの人工的因子によらない熱帯の風土条件を重要な因子と考えている。ガーナでも近代化が進んだ Coastal Area で Au 抗原陽性率が低いこと，アメリカ在住の黒人間で低いという成績[4]はこの考えを示唆している。さらに，アフリカ産のチンパンジー，アフリカみどりざるなどの霊長類で Au 抗原が増殖し継代できること[20]，アフリカ産のチンパンジーから飼育者が感染し肝炎をおこしたとの報告[21]は，ジャングル内での人間と動物間での Au 抗原の伝播が推察できて興味深い。

結　　論

1970 年 3 月より 1971 年 2 月までの間に，ガーナ各地から採取した 269 例の黄疸症患者を含む 4,789 名のガーナ人血清について HI 抗体測定による黄熱病の血清診断と Au 抗原の検出を I. E. S. と一部 CF，電顕で行ない次の成績を得た。

1. 黄熱病ウィルスによる感染と考えられる黄疸症例はわずか 9 例 (3.4%) であった。

2. 269 名の黄疸症例の Au 抗原陽性率は I. E. S. で 33..7%，CF で 53.9% であった。

14 才以下の患者群からは I. E. S. で 58.5%，CF で 71.8% から Au 抗原が検出された。

3. 非黄疸症群 4,520 名の Au 抗原陽性率は，入院患者群で 6.3%，健康者群で 6.2%，献血者群で 5.1% であった。Au 抗原陽性率は年令により差がみられた。すなわち 0～2 才児 (1.3%) で低く，10 代までは年令とともに上昇し 10% 台を示し，10 代，20 代で高く，以後除々に下降し，45 才以上では 3.1% であった。

4. Au 抗原陽性率は地域差がみられ，Coastal Area で低く，Forest，Savanna Area で高かった。部属は地域差ほど重要な因子ではないようであった。

5. 男性の Au 抗原陽性率が女性よりも高かった。

これらの成績にもとづいて，ガーナにおけるウィルス性肝炎と Au 抗原との関係，Au 抗原の伝播に関する考察を試みた。

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[I-5]

ENTEROVIRUS SPECTRUM OF HEALTHY, NON-DIARRHOEAL CHILDREN (0-15 YEARS) IN THE GREATER ACCRA REGION BETWEEN AUGUST, 1971 AND JULY, 1972

By

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Introduction

Enteroviruses are known to cause a variety of clinical syndromes of public health importance both in man and in animals. Such syndromes include febrile illness, exanthems, respiratory diseases, diarrhoeas, conjunctivities, herpangina, myocarditis, pericarditis, Bornholm disease, CNS and locomotor system disorders, etc. To the last syndrome, i.e. CNS and locomotor disorders belong the much dreaded disease, paralytic poliomyelitis, which in the past has ravaged many parts of the world. However, since the introduction of inactivated poliovirus vaccine in 1955, and especially after 1959 when live attenuated vaccines became available on a large scale, the incidence of poliomyelitis has fallen to insignificant proportions in North America, parts of Europe and several other countries (Memorandum, Bull. WHO., 1969; Cockburn and Drozdov, 1970). However, in large areas of Africa, Asia and Latin America, where the most susceptible persons are predominantly children under 5 years of age, the incidence of poliomyelitis appears to have been increasing disconcertingly (WHO) Chronicle, 1968).

Information about the poliomyelitis situation in Ghana in the years preceeding 1969 has been scanty. Between 1951 and 1955, 59 poliomyelitis cases were recorded. There were no reports of the disease from 1956 to 1960. In 1961 through 1965, however, only 29 cases were recorded. In the three years that followed, there were 7 cases recorded in 1966, 5 in 1967 and 10 cases recorded in 1968 (Cockburn and Drozdov, 1970). In 1969, 134 cases were recorded. The number of cases reported in the first half of 1970, that is, from January to August, was 72, the highest number ever recorded in this country so far for one half of a year. In September, the same year, an additional 13 cases were reported giving for the period January to September, a total of 85 cases. A higher number of cases than those given above should be expected, since these figures represented only those cases reported to Government Hospitals. Thus, cases reported to Private Hospitals, Mission Hospitals and Health Centres have not been included in these medical statistics. Although these medical statistics did not provide the actual number of cases that occurred in the country during the previous years, there could be no doubt that with better reporting and recording of cases, the incidence of the disease in this country must be found to be on the increase.

The aim of this survey is firstly to determine which poliovirus type is more prevalent among the youth of this country at the present time and secondly to ascertain which proportion of this poliovirus type is virulent and therefore poses a threat to the community. The survey also aims at determining the monthly or seasonal incidence of enteroviruses among the groups studied. The findings of this survey should help in the planning and subsequent launching of a continuing poliomyelitis vaccination programme aimed primarily at protecting the most susceptible group in the community.

Materials and Methods

Specimens: Single Rectal swab or stool specimens were obtained from 741 non-diarrhoeal patients between August 1971 and July 1972. The patients included children between the ages of 2 months and 15 years attending the Korle Bu Teaching Hospital and any one of the following three Polyclinics in the Greater Accra Region: (*a*) The Ussher Clinic, (*b*) The Kaneshie Urban Health Centre and (*c*) The Danfa Rural Health Centre.

Stool and rectal swab specimens were collected in 3 ml. Transport Medium (LE-1200) containing 1200 units of Penicillin/ml. and 1200 gm. of Streptomycin and either extracted immediately or stored at—20°C. Prior to testing, the frozen samples were thawed, agitated for thorough mixing and then spun down at 3,000 rpm. for 30 minutes in a cold centrifuge. The clarified supernatant fluid was inoculated immediately onto tissue culture or stored at — 20°C until used.

Tissue Culture: HEp.2 cells were grown on Eagle's MEM containing 10% calf serum and maintained in Eagle's MEM containing 5% calf serum and antibiotics.

Isolation: Two to four HEp-2 tissue culture tubes were each inoculated with 0.1 ml. or 0.2 ml. amounts of the treated rectal swab or stool specimen. Prior to the inoculation, the growth medium was removed and the cells washed 3 times with PBS. After spreading the specimen over the entire surface of the culture, the tubes were stoppered and incubated stationary at 37°C for 1 hour. Thereafter, the suspensions were removed, the cell cultures washed 3 times with phosphate buffered saline (PBS) and 2 ml. of maintenance medium pipetted onto the cells. All cultures were incubated stationary at 37°C and readings for cytopathogenic effect (CPE) were made periodically. The harvests from tubes of the same sample showing CPE of about 70–90% of cells were pooled after overnight storage at –20°C. Cultures without CPE were incubated for 7 days. In addition to the primary inoculum, two additional passages were made before any specimen was reported as negative.

All isolated viruses were titrated and their titres calculated by the Reed and Muench method (Reed and Muench, 1938).

Identification of Isolates: Typing was carried out on 3rd passage isolates by the tube CPE neutralization method, in which 0.25 ml. of virus suspension having a concentration of 100 $TCID_{50}$ was mixed with an equal volume of each of 13 WHO Schmidt Intersecting Serum Pools containing the majority of the ECHO-viruses, Poliovirus types 1, 2 and 3, Coxsackieviruses A7, A9 and A16, and Coxsackieviruses B1 through B6. The virus-serum mixtures were incubated in a water bath at 37°C for 1 hour, 0.2ml of each of the 13 virus-serum mixtures being then pipetted into each of 2–4 culture tubes and incubated stationary at 37°C. Each virus isolate was titrated in the same test and the typing repeated if too little or too much of the virus was used in the test. The final scoring was done on the 7th post-inoculation day. All identified Poliomyelitis viruses were again tested against 20 Neutralizing Units of antisera to polio type 1 (Mahoney), polio type 2 (MEF-1) and polio type 3 (Saukett) in a check-board neutralization test to determine the neutralization indices (Addy, 1968; Addy, 1970).

Determination of the rct/40: The determination of the rct/40 marker test was carried out according to the method recommended by Domok *et al.* (1961). The T marker test was used to determine the growth of poliomyelitis viruses at 40°C. Attenuated strains fail to grow at such an elevated temperature and are designated T^-, whereas virulent strains grow effectively at this temperature and are designated T^+.

Results

A total of 741 stool/rectal swab samples were collected and divided according to the age of the donors into the following age groups: 0–4 years, 5–9 years and 10–15 years. From these samples, as shown in Table 1, 183 enteroviruses were isolated giving a 24.69 percentage isolation. Only 68 (36.67%) of these isolates could be serotyped; the remaining 115 (63.33%) could not be identified using the WHO Schmidt Interesecting Serum Pools. As can be seen again from Table 1, 47.13% of the isolates were from samples collected from children within the age group 0–4 years, 28.57% from children within the age group 5–9 years and only 11.53% were obtained from children within the age group 10–15 years. Table 2 shows that out of a total of 47 poliovirus type 1 strains identified, 23 (48.93%) were from the age group 0–4 years, 11 (23.40%) from the age group 5–9 years and 13 (27.65%) from the age group 10–15 years. The relative distribution of Poliovirus types 1, 2 and 3 shown in Fig. 1.

Apart from the polioviruses identified in this study, 14 Coxsackie B viruses and 6 echoviruses could be identified. Their distribution according to age-group and serotype is as set out in Table 2.

Some 12 samples were found to contain a mixture of 2 or 3 enteroviruses. Seven out of the 12 were found to contain a mixture of Poliovirus type 1 and Cox B5, two samples yielded Poliovirus type 1, Coxsackie B5 and

TABLE 1. *Isolation pattern of identified and Non-identified enteroviruses from faeces of Non-diarrhoeal children (August, 1971—July, 1972)*

Age Group	Abs. number of samples	Positive Isolates		Typed Isolates		Non Typeable Isolates	
		Abs. numbers	% of sample	Abs. numbers	% of all Isolates	Abs. numbers	% of all isolates
0— 4	157	74	47.13	29	39.19	45	60.81
5— 9	245	70	28.57	18	25.71	52	74.29
10—15	339	39	11.53	21	53.84	18	46.16
Totals	741	183	24.69	68	36.67	115	63.33

TABLE 2. *Distribution of Enteroviruses (Polio, Coxsackie, Echo.). Isolated from Non-diarrhoeal children (August, 1971—July, 1972)*

Age Group	Total Number of Positive Isolates									
	Poliomyelitis			Coxsackie			Echoviruses			
	P. 1	P. 2	P. 3	B. 1	B. 4	B. 5	E. 3	E. 11	E. 12	E. 24
0— 4	23	0	0	0	2	3	0	0	0	1
5— 9	11	1	0	1	1	2	0	1	0	1
10—15	13	0	0	2	0	3	1	0	1	1
Total	47	1	0	3	3	8	1	1	1	3

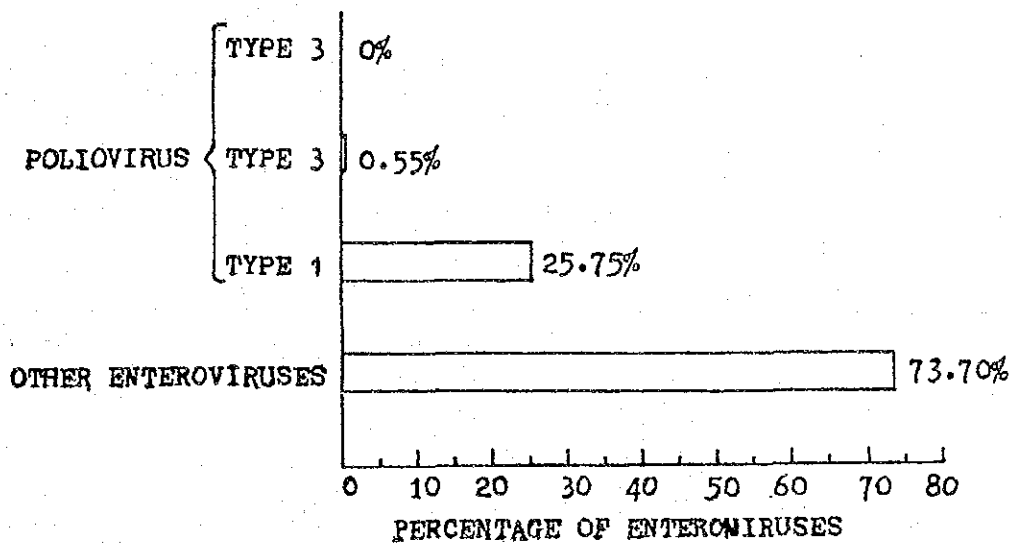

FIG. 1: *Relative Distribution of Poliovirus Types 1, 2, & 3 and other enteroviruses in faces of non-diarrhoeal children.*

ECHO 12. The remaining 3 of the 12 'mixed' samples were found to contain the following enterovirus mixtures: Poliovirus type 1 and ECHO 3, Poliovirus type 1 and Coxsackie B1 and Poliovirus type 1 and ECHO 11.

Monthly Distribution of Enteroviruses:

In Figure 2, is summarized graphically, the percentage of enteroviruses isolated per month of the study period lasting from August 1971 through July 1972. Apart from the months of December (34.42%), January (46.15%) and February (50.00%) which showed rather high isolation rates, the other months in the study period gave rates ranging between 13.11% and 25.00%.

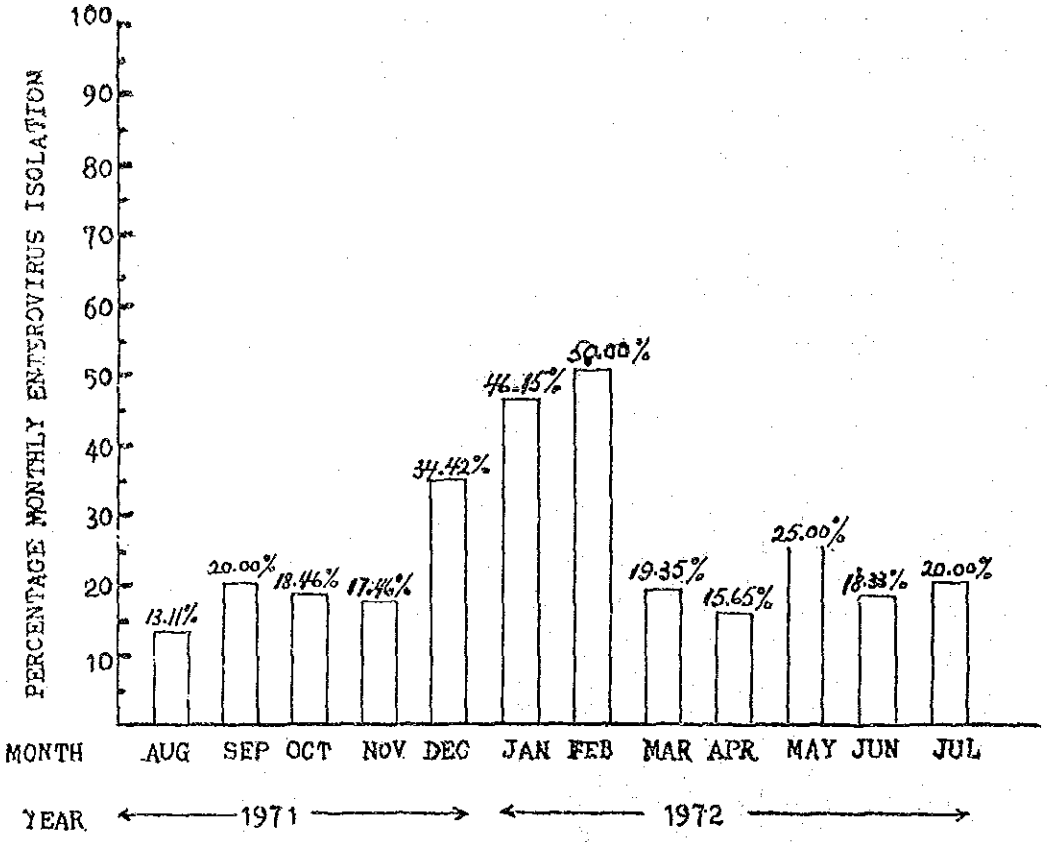

FIG. 2: *Monthly isolation of enterivoruses during the period, August, 1971 through July, 1972 from healthy non-diarrheal children.*

TABLE 3. *Distribution by age groups of virulent, intermediate and avirulent polioviruses*

Age Groups	Genetic T (rct/40) Marker Test			Strains Not Tested
	T+	T+	T—	
0 — 4	17 (58.62%)	1	2	4
5 — 9	5 (17.24%)	1	2	4*
10 — 15	7 (24.86%)	1	2	2
Totals/% Age ...	29 (76.34)%	3 (7.88%)	6 (15.88%)	10

* 1 *Poliovirus Type 2 Included.*

Genetic Marker, rct/40:

Thirty-eight out of a total of 48 Poliovirus strains identified in this study were tested for the rct/40 (T) marker. The results of the test are shown in Table 3. From the results 29 (76.34%) out of 38 Polio-virus type 1 strains tested were T^+ ($rct/40^+$); 3 (7.88%) were T^+ ($rct/40^\pm$) i.e. intermedidiate and 6 (15.88%) were T^- ($rct/40^-$). The age distribution of poliovirus type 1 with the T^+ marker (virulence) is as follows: 17 (58.62%) were isolated from children in the age-group 0–4 years, 5 (17.24%) in the age group 5–9 years and 7 (24.86%) in the age group 10–15 years. Polioviruses not tested for the rct/40 marker included 9 Polioviruses type 1 and 1 Poliovirus type 2.

Discussion

In our survey, which covered an entire year, 183 enteric viruses were isolated in HEp-2

cell line, from a total of 741 stool/rectal swab specimens collected from children between the ages of 2 months and 15 years in the Greater Accra Region. The rate of enteric virus isolation was 24.69% for all age groups combined. This figure compares favourably with the 26% enteric virus isolation rate obtained by Lee *et al.*, (1965).

Since HEp-2 alone could give 24.69% compared with the 26% yield by four different cell lines employed by Lee *et. al.*, 1965, the proportion of enteric viruses which did not grow on HEp-2 should not at all be great. However, it is recommended by Lee *et. al.*, (1965) that in a survey of this nature, more than one cell line should be used for primary virus isolation attempts.

From the data obtained in this survey, it could be seen that only 36.67% of the isolates could be serotyped using the WHO Schmidt Intersecting Serum Pools; the remaining 63.33% could not be serotyped. Contrary to the proportion of isolates serogically identified by us, Grinstein *et. al.*, (1970), could readily serotype only 5% (772 out of 12,855 isolates) of their virus isolates. This frequently high percentage of unidentified isolates can be attributed to the possible occurrence of small aggregates of virus particles in the suspension and not to the presence of virus particles that are resistant to neutralization by immune serum. (Wallis and Melnick, 1967).

Of the 48 polioviruses isolated in this study, 47 (97.92%) were identified as poliovirus type 1 and only 1 (2.08%) as poliovirus types 2. No poliovirus type 3 was isolated. A report from the Committee on Typing of the National Foundation for Infantile Paralysis, (1953,) indicated that prior to the introduction of mass scale poliomyelitis vaccination in 1955, 196 strains of polioviruses isolated between 1909 and 1951 in 19 countries in different parts of the world, 82% belonged to type 1, 10% to type 2 and 8% to type 3. This finding was substantiated by Domok and Molnar in 1961. It has also been found that in areas without adequate vaccination programmes poliovirus type 1 has been responsible for much of the disease of the Central nervous system (CNS) (Memorandum, Bull. Wld. Hlth Org., 1969). However, in areas with good vaccination programmes equal numbers of the three types have been isolated from patients with other diseases or no illness (Cockburn and Drozdov, 1970). After the use of poliovaccines became widespread, several reports showed that as the incidence of poliomyelitis decreased the proportion of cases caused by poliovirus type 3 increased (Cockburn, 1962; WHO Memorandum, 1969). In Ghana, no mass vaccination against poliomyelitis has ever been undertaken. We are therefore, yet to study this situation in Ghana. Our inability in this study to isolate any poliovirus type 3 does not preclude the existence of type 3 poliovirus in the Ghanaian population. However, Lee *et al.*, (1965), reporting from Singapore, where mass vaccination had been undertaken, could not observe this trend. It is most likely, that the population Lee and associates investigated were inadequately vaccinated, hence the discrepancy.

Since in areas where poliovaccines are either used on an inadequate scale or not at all, type 1 poliovirus is still responsible for about 80% of the paralytic cases (WHO Memorandum, 1969, Domok and Molnar, 1961), it would be interesting to note that of the 68 enteroviruses serologically identified in our study, poliovirus type 1 constituted about 70%, 48.93% of which were isolated from children in the age group 0–4 years. Results of the rct/40 marker test showed that 76.34% of the poliovirus type 1 identified in this study were virulent strains, 58% of which were from children in the age group 0–4 years. From the discussions of our findings so far, it would not be far-fetched if it was postulated that poliovirus type 1 should be responsible, at present, for the majority of paralytic poliomyelitis in this country and that the most susceptible group is the 0–4 years age-group in our community. In a survey conducted by the Poliomyelitis Commission of the Western Region Region Ministry of Health, Nigeria (Bull. WHO., 1966), on the incidence of poliomyelitis in Nigeria, it was found that the maximum incidence of the disease was in children between 1 and 2 years of age; the disease was progressively less common in older children and rare in children above the age of 7 years.

Although the design of our survey departs somewhat from that of the Nigerian survey, yet the findings of both surveys indicate that the younger age-group is most susceptible to poliomyelitis. A continuing vaccination programme for this age-group is therefore called for.

It is further interesting to note that in this study a correlation of the months of specimen

collection and enterovirus isolation could be established. It was found that the incidence of enteroviruses in the community was at its height between December and February. Paul, (1955) and Bodian and Horstmann, (1965), indicated that in tropical countries, poliomyelitis occurs more or less uniformly throughout the year, with only slight concentration in the hottest months. Since the hottest months of the year in Ghana are the months of December, January and February, our finding must be consistent with that of Paul, (1955) and Bodian and Horstmann, (1965). It is indeed during the hottest months of the year that children and adults alike go bathing in ponds, lagoons and rivers, not excluding the sea. Since experiments performed by various researchers (Gard, 1940; Melnick, 1947; Kelley, 1953; Melnick, 1957; Matcalf and Stiles, 1968; Grinstein, Melnick and Wallis, 1970; Addy and Otatsume, unpublished) have proved that rivers, lakes, lagoons, etc., do harbour large quantities of enteric viruses including enteroviruses and the causative agent of infections hepatitis, which organisms are very resistant to chlorination and other adverse environmental conditions there is the likelihood therefore that the rate of infection with these viruses would be high during this period of the year and hence the high rate of enteric virus isolation in December, January and February observed in this study.

Another possible factor which might have contributed to the high rate of enteric virus isolation in December through February is the great numbers of house-flies which can be sources of contamination of food during this period. (Sabin and Ward, 1941; Trask, Paul and Melnick, 1943; Ward, Melnick and Horstmann, 1945; Gear, 1952).

On the strength of our survey findings, we would like to recommend the following:–

1. That plans should be made to ensure prompt control of poliomyelities as soon as soon as an outbreak is recognized.

Such planning should include (*a*) arrangements for isolation and rapid identification of the virus type responsible for the outbreak of poliomyelitis; (*b*) the launching of a continuing rather than a mass Poliomyelitis vaccination programme, aimed primarily at protecting the most susceptible group, i.e. children within the age group 0-4 years, in the community and (*c*) arrangements for periodic vaccine potency testing.

2. That Sabin's live attenuated polyvalent vaccine should be used for the continuing vaccination programme. In the event of an epidemic, the monovalent vaccine (Sabin) of the virus type responsible for the epidemic should be used accompanied by injections of Salk's polyvalent inactivated vaccine.

Summary

Between August 1971 and July 1972, 183 enteric viruses were isolated from a total of 741 faecal/rectal samples collected from children aged 2 months—15 years in the Greater Accra Region. Of these 68 (36.67%) were serotyped as belonging to Polio 1 (47) Polio 2 (1), Cox B-1 (3), Cox B-4 (3), Cox B-5 (8), ECH-3 (1), ECHO-11 (1), ECHO-12 (1) and ECHO-24 (3). Of the 68 enteroviruses identified, 70% were Poliovirus type 1, 48.93% of which were from children in the 0-4 years age group. 76.34% of the Poliovirus type 1 were found to be virulent strains (T^+). Of these, 58 were isolated from children in the age group 0-4 years. The survey showed that December through February are the months of maximum enterovirus isolation.

A number of suggestions emanating from the results of the survey were made.

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[I-6] **LEPTOSPIROSIS IN GHANA**

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Leptospirosis is one of the comon infectious diseases in tropical countries, and it is important to differentiate the severe form of this disease, from other diseases with jaundice such as yellow fever or infectious hepatitis. However, there are few reports on leptospirosis in West Africa except a series of papers by van Riel *et al*, (1953–56) in Zaire, former Belgian Congo, and in Ghana we are aware of only the serological survey reported by Rothstein in 1964. It may, therefore, be interesting to report on a more recent investigation on human cases of leptospirosis in this country.

Materials and Methods

SERA: The majority of serum samples were collected from Agogo Hospital in Ashanti Region and Adidome Hospital in the Volta Region. As far as possible, paired sera were requested, that is acute and convalescent serum samples.

ANTIGEN: Live Leptospires were used as antigens. They were cultivated on Korthof's medium for two or three weeks at Room Temperature (26°C—30°C). Fifteen serogroups of Leptospires recommended by the WHO Expert Committee (1967) on Leptospirosis were tested against each serum. The names of strains and serogroups are as listed below:

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* (Wolffii group).
12. Akiyama B (Hebdomadis group).
13. Van Tienen* (Bataviae group).
14. Hyos (Tarassovi group).
15. RS 173 (Semaranga group).

**Reference strains recommended by WHO Expert Group.*

Agglutination Test: The microscopic agglutination test technique was used. The original technique was described in detail by Wolff in 1954 and this was later modified by Yamamoto in 1957. The latter technique was

used in this investigation. The differences between the two methods are that in the former, serum dilutions are made with 0.85 per cent saline and incubated at 32°C. In the latter, serum is diluted with Korthof's medium and kept at 37°C. The material is examined at low power magnification (x150) and by darkfield illumination.

Reading of Results: It is recommended by the WHO Expert Group that the end-point in the positive serum-antigen mixture be determined by the presence of agglutination masses with less than 50 per cent of free motile leptospirae as compared with negative control mixtures. We also regarded the end-titre of 1 in 300 and over as significantly positive.

Screening Test: For the purpose of saving time and materials an initial screening method was used. A single serum dilution of 1 in 100 for each specimen was examined. If positive against any strain, the neat serum was then diluted usually from 1 in 10 to 1 in 3,000 and re-examined against these suspected strains.

Results

We examined a total of 137 sera, consisting of 38 paired and 61 single specimens. The former represented samples from acute and convalescent stages and the latter only from the acute stage of the illness; therefore the actual number of cases totalled 99.

Table 1 shows the sources of the suspected cases of Leptospirosis and related serological results. Of the 38 cases of paired sera, 11 gave positive reactions and the incidence was rated as nearly 29 per cent. Of the 61 cases of un-paired sera, 10 positive reactions were obtained giving an approximate incidence rate of 16 per cent, i.e. about one half as compared with the paired sera cases.

Table 2 presents the results tabulated according to clinical diagnosis or presenting symptom, most of the cases having been diagnosed as jaundice or infectious hepatitis.

Table 3 indicates the Leptospiral serogroups involved and the end titres of sera in positive cases. Owing to shared antigen reactions, many cases showed high titres against more than one serogroup, but the dominant reaction was usually obvious in such cases. With the exception of case No. 8, all the paired sera showed a rise in reactivity during the convalescent stage confirming that the true clinical diagnosis was in fact leptospirosis

It will be noted that seven different kinds of Leptospiral serogroups were apparently involved in the cases which were reported at Agogo Hospital. On the other hand, only the Icterohaemorrhagiae group was involved in the Adidome cases. Of the 21 positive cases, 7 were of the Icterohaemorrhagiae group, 4 of the Tarassovi group, 3 each of

TABLE 1. *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 2. *Correlation between clinically suspected leptospirosis and serological results*

Clinical Diagnonsis or Presenting Symptom	PAIRED SERA		SINGLE SERUM	
	Number of Cases	Number Positive	Number of Cases	Number Positive
Infectious Hepatitis	17	7	23	2
Jaundice	20	4	31	7
Meningitis	—	—	2	0
Yellow Fever	—	—	1	0
Haematuria	—	—	1	0
Not Stated	1	0	3	1

TABLE 3. *Leptospiral Serogroups and Antibody-Titre of Positive Cases*

Case No.	Name of Hospital	Clinical Diagnosis Presenting symtom	First Specimen (Acute)	Second Specimen (Conval)
1.	Agogo	Infectious Hepatitis	Negative	Autumnalis X 300 Bataviae X 100
2.	Agogo	Jaundice	Cynopteri X 100, Pomona X 100	Cynopteri X 300 Pomona X 100
3.	Agogo	Jaundice	Negative	Ballum X 300
4.	Agogo	Infectious Hepatitis	Bataviae X 1000, Australis & Icterohaemorrhagiae X 100	Bataviae X 1000 Canicola X 300 Australis, Icterohaemorrhagie & Grippotyphosa X 100
5.	Agogo	Infectious Hepatitis	Negative	Bataviae X 300 Icterohaemorrhagiae & Tarassovi X 100
6.	Agogo	Infectious Hepatitis	Tarassovi X 300	Tarassovi X 1000
7.	Agogo	Infectious Hepatitis	Negative	Canicola X 300 Icterohaemorrhagiae X 100
8.	Agogo	Infectious Hepatitis	Tarassovi X 300	Tarassovi X 100
9.	Agogo	Infectious Hepatitis	Negative	Icterohaemorrhagiae X 300
10.	Adidome... ...	Jaundice	Negative	Icterohaemorrhagiae X 300
11.	Adidome... ...	Jaundice	Negative	Icterohaemorrhagiae X 300
12.	Agogo	Infectious Hepatitis	Canicola X 300	—
13.	Agogo	Jaundice	Tarassovi X 300	—
14.	Agogo	Jaundice	Autumanalis X 300 Icetero-haemorrhagiae, Australis & Pomona X 100	—
15.	Agogo	Jaundice	Bataviae X 1000.	—
16.	Agogo	Jaundice	Canicola X 300 Australis, Bataviae & Grippotyphosa X 100	—
17.	Adidome... ...	Jaundice	Icterohaemorrhagiae X 300	—
18.	Adidome... ...	Jaundice	Icterohaemorrhagiae X 300	—
19.	Adidome... ...	Jaundice	Icterohaemorrhagiae X 1000	—
20.	Korle Bu ...	Not Stated	Icterohaemorrhagiae X 3000	—
21.	Legon	Infectious Hepatitis Hepatitis	Tarassovi X 1000, Grippotyphosa X 300, Icterohaemorrhagiae & Bataviae X 100	—

Note: The time interval between the acute and convalescent samples was between 1–4 weeks.

the Canicola group and of the Bataviae group, 2 of the Autumnalis group and one each of the Cynopteri group and the Ballum group.

Discussion

It is to be expected that many kinds of Leptospira serotypes will be prevalent in tropical Africa. In fact, previous reports (Van Riel *et al*, 1953, 1955, 1956) have indicated that in Zaire, former Belgian Congo 7 serogroups were present among the oxen and the dogs and 5 of them were also found among the human patients. In Ghana, a previous report (Rothstein, 1964) indicated

that 65 of the 117 cases hospitalized as infectious hepatitis gave positive reactions against Leptospiral antigents. However, the specific serotypes of the Leptospira were not established because pooled antigens, consisting of plural serotypes, were used, so this study is perhaps the first attempt to define the specific aetiologic types of Leptospires causing human infection in this country.

According to the WHO Report (1967), the number of independent serotypes of Leptospires now exceeds 120. It is, therefore, impossible to use all these serotypes in an investigation such as ours and it is therefore advisable to utilize reference strains of serogroups representing serotypes which cross agglutinate to high titre with each other. Our investigation was carried out with 15 such strains as recommended by the WHO Expert Group. For the reason mentioned above, we were not able to define the serotype in each of the positive cases but were able to determine the serogroups. The next move will be to isolate the causal micro-organisms from the human patients or animals which form the reservoir and thus to identify the serotypes.

From the results in Table 3, it appears that a variety of serotypes were involved in the cases derived from Agogo Hospital. This hospital is situated in the moist "Semi-deciduous Forest" zone. Adidome Hospital, on the other hand, is situated in the "Guinea Savannah Woodland" zone and the remaining two hospitals in the coastal "Grassland" belt. It is interesting to note that virtually only one serotype was involved in the cases outside the forest zone.

We have also gained an impression that there was some difference among the positive cases which belonged to the same serogroup. For instance, of the 4 positive cases of Tarassovi group, Cases No. 6, 8, and 13 reacted against Tarassovi group antigen only, but many cross-reactions were found with Case No. 21. A similar reaction was noted in the Canicola group and the Bataviae group. This observation may, not necessarily, be due to cross-reactions because no cross-reactions are known to exist among some of the combinations we found positive (Wolff, 1954). It is, therefore, possible that some cases may represent either multiple infections with plural leptospiral serotypes or infection by some new unidentified serotypes.

In considering the results from the paired sera, a rise in reactivity of the convalescent serum sample was regarded as evidence of present infection of Leptospirosis. With the un-paired sera, however, the high anti-body titres could have implied either present infection in some cases or residual titres due to past infection in other cases. Of the 11 positive cases with paired sera, 7 cases showed negative reactivity with the acute samples due to the fact that in Leptospirosis the specific antibody may not appear in the blood until about a week after the onset of clinical symptoms. The lower incidence rate from single serum cases as compared with paired sera cases was possibly due to the same reason.

Summary

A total of 99 cases, mainly diagnosed as infectious hepatitis or jaundice, providing 38 paired sera and 61 single sera were examined by microscopic leptospiral agglutination test using 15 strains of live leptospirae. Of the former 38 cases, 11 (28.9%) gave positive reactions, and of the latter 61 cases, 10 (16.4%) were positive.

Leptospiral serogroups of the positive cases are as follows: Icterohaemorrhagiae, Tarassovi, Conicola, Bataviae, Autumnalis, Cynopteri and Ballum. It was found that there is evidence of more variety of Leptospirae in the forest area than in the savannah area.

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[1-7] # THE SICKLE-CELL ERYTHROCYTE MEMBRANE AND THE TRANSPORT OF CATIONS

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The erythrocyte maintains an osmotic but not an electrochemical equilibrium with its environment. Passive and active transport of ions maintains the osmotic equilibrium.

In the present study intraerythrocyte [Na] and [K] have been determined for 47 normal Negroid Ghanaians (with haemoglobin AA, Hb AA) and 31 sickle-cell anaemic subjects (Hb SS). The cell water content has also been measured for 11 subjects in each group. For comparison, the [Na] and [K] have been measured for 8 Caucasian erythrocytes.

The erythrocytes were centrifuged from the plasma (1000 x g, 20min.), washed three times in 4 volumes of buffered, isotonic $MgCl_2$ (20mM Tris-HCl, pH 7.4), and haemolysed in deionized, distilled water for cation determination by flame photometry (EEL Model 150). Erythrocyte water was determined on thoroughly packed cells, dried at 105°C to constant weight.

The erythrocyte concentration of sodium was 40% higher and potassium 10% lower in Hb SS than in Ghanaian Hb AA cells.

Hb SS: [Na] = 20.62 ± 0.92, [K] = 99.07 ± 1.19;
Hb AA: [Na] = 13.96 ± 0.76, [K] = 89.37 ± 2.82 (mean + SEM mEq/L cell water).
The differences were statistically significant: Na, $P < 0.001$; K, $P < 0.01$.
The cell water (corrected for trapped plasma) was identical for both cell types:
Hb SS 66.27 ± 0.55% per gram erythrocyte, Hb AA 66.85 ± 0.46% per gram erythrocyte ($P > 0.5$).
Normal caucasian (resident in Ghana) erythrocyte contained 40 to 50% less sodium but about the same potassium concentration as Ghanaian Hb AA cells.
Caucasian [Na] = 9.10 ± 0.70 ($P < 0.001$); [K] = 95.34 ± 1.47 ($P < 0.1$).

The high intraerythrocyte sodium observed in Negroes refutes the claim by Balfe et al (1) that this range of [Na] is a hereditary abnormality. However the plasma concentrations of these cations compared very closely in all three groups of subjects ($P > 0.5$).

Passive efflux of potassium was studied by suspending Negroid Hb AA and Hb SS erythrocytes in buffered, isotonic sucrose and determining intracellular potassium changes and its appearance in the medium with time. The rate of passive efflux of potassium was about 15 times faster in Hb SS cells during the first 120 minutes of the process. Active sodium transport in the cells was also studied according to the method of Post and Jolly (2). Hb SS cells actively transported sodium twice faster than Hb AA cells, in accordance with higher glycolysis observed in sickle-cells (3,4).

Light microscopy and cell count show that not less than 85% of the Hb SS erythrocytes under the conditions of the experiments appeared spherical as observed with Hb AA cells. Electron microscopic examination of ultra-thin sections of cells fixed in buffered glutaraldehyde (pH 7.2), under oxygenated conditions, showed marked discontinuities in the Hb SS erythrocyte membrane. In some sections a unit membrane was not detectable even at higher magnification

This membrane aberration in Hb SS cells results from asymmetrical protrusions of polymerized haemoglobin in sickling (5), leading to irregular loss of membrane plasticity and perhaps localized membrane reorganization. Most likely, it has resulted from the sickling - unsickling cycles which Hb SS erythrocytes undergo during their life span. We suggest that this membrane alteration augments passive cation diffusion such that active transport is enhanced, establishing a new cation equilibrium in sickle erythrocytes.

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[I-8]

ALTERATIONS IN MEMBRANE STRUCTURE AND TRANSPORT PROPERTIES IN SICKLE CELL ERYTHROCYTES*

By

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Abstract

The erythrocyte is delimited by a membrane which is highly permeable to water and ions such as chloride and bicarbonate but sparingly permeable to cations of comparable size such as potassium and sodium. As a matter of homoestatic necessity the cell maintains an osmotic but not an electrochemical equilibrium with its environment. The osmotic equilibrium is maintained by the combined effects of passive diffusion and active transport of ions. In the present study, the intracellular concentrations of sodium and potassium have been determined for 47 normal negroid Ghanaians (with haemoglobin AA, Hb AA) and 31 sickle cell anaemic csubjects (Hb SS). The cell water content has also been measured for 11 subjects in each group. For comparison, the sodium and potassium concentrations have been measured for 8 caucasian erythrocytes. The results (expressed as mEq per litre cell water) show that the Hb SS erythrocyte has about 40% higher intracellular sodium and about 10% lower intracellular potassium: mean $[Na]_i$ $=20.62 \pm 0.92$ (SEM); $[K]_i = 89.37 \pm 2.82$; than Hb AA cells; mean $[Na]_i = 13.96 \pm 0.76$ $[K]_i = 99.07 \pm 1.19$. The difference was statistically significant: Na, $P < 0.001$ and K, $P < 0.01$

The cell water content (corrected for trapped medium) did not show any difference in the two cell types: Hb SS 66.27 $\pm$ 0.55 per cent per gm erythrocyte and Hb AA 66.85 $\pm$ per cent per gm erythrocyte ($P > 0.5$). As reported earlier, the normal caucasian erythrocyte contains about 40–50% less sodium and about the same concentration of potassium: $[Na]_i = 9.10 \pm 0.70$ ($P < 0.001$) and $[K]_i = 95.34 + 1.47$ ($P < 0.1$) compared to normal negroid (Ghanaian) erythrocyte. However, the plasma concentrations of these cations compared very closely in all three groups of subjects ($P > 0.5$). Comparison of the passive and active transport in Hb SS and Hb AA cells show that passive potassium efflux in cells suspended in isotonic sucrose (buffered with Tris-HCl to pH 7.4) is about 15 times faster in former cells during the initial period of the process. Also the active sodium transport is twice as fast in the Hb SS cell compared to the Hb AA cells. Light microscopy and cell count show that not less than 85% of the Hb SS cells under the conditions of the experiments remain in an apparently unsickled state. Electron microscopic examination of the cell membrane of Hb SS and Hb AA shows marked discontinuities and asymmetrical protrusions in the Hb SS membranes (as a result of Hb polymerization in sickling). This membrane aberration leads to "pits" or "holes" in the membrane; to irregular loss of membrane plasticity and perhaps localize, membrane reorganization. This condition, most likely, has resulted from the sickling—unsickling cycles which the Hb SS cell undergoes during its life span. We suggest that this membrane aberration leads to augmented passive diffusion of sodium and potassium. To maintain the operations of feedback mechanisms the active transport of sodium should be enhanced. A new state of cation equilibrium is thus established in the Hb SS cells at which the rate of passive diffusion and active transport of cations are pitched at a higher level, and hence the intra-erythrocate sodium and potassium concentration at a different equilibrium.

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*Presented at the Fifth Scientific Meeting of the Research Committee, University of Ghana Medical School, 24th March, 1973. The authors are very grateful to Dr. F. I. D. Konotey-Ahulu for allowing these studies to be done on his patients in the sickle-cell clinic, Department of Medicine, Korle Bu Teaching Hospital, Accra.

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[I-9]

AN EPIDEMIOLOGICAL INVESTIGATION ON VIRAL DISEASES IN GHANA

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An Epidemiological Investigation on Viral Diseases in Ghana

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Introduction

One of the most important problems of the developing countries is undoubtedly that of the health of the people. It is not unusual that the average life span of human beings is forties in these developing countries which are located mainly in tropical and subtropical zones. Ghana, a country in West Africa was also once called as "the cemetery of the whites", and was reckoned as one of the most unhealthy areas in the world. The average life span of the people in Ghana is said to be about 42 years. This low average is attributable to high mortality rate among infants. It is not rare that persons who could break through the pre-carious period lived to a ripe old age of eighties. Generally, the main cause for infants' deaths is considered to be infectious diseases of various kinds. These include viral diseases such as measles, poliomyelitis, yellow fever, smallpox and infectious hepatitis; bacterial diseases such as dysentery, typhoid fever, cholera which was first imported into West Africa in 1970 and seemed to have settled there, epidemic cerebrospinal menigitis and tuberculosis, and various diseases caused by protozoa such as malaria and amoebic dysentery, etc. Probably there may be some other infectious diseases that are yet to be detected. The mortality rate by these infectious diseases seems to have reached almost its limit, by the concominant effect of unfavorable factors such as malnutrition, severe climatical conditions, poor environmental sanitation and limited medical cares.

If the health of a people is considered to be the foundation of a country, medical doctors who are charged with its administration and guidance are also considered to be the foundation of the country. The total number of doctors in Ghana between September 1969 to July 1971 was about 550. Since the total population of this country is about 8.5 million, the number of doctors per 100,000 persons is only 6.5 This figure is less than 1/10 to 1/20 of that of advanced

* The present studies were performed as a part of the Medical Cooperation Projects between the Governments of Ghana and Japan, during the period between the 1st October 1969 and the 21st July 1971, when the author was on his duty as a visiting professor at the Department of Microbiology, University of Ghana Medical School, Accra, Ghana.

nations. Moreover, about 1/3 of the limited number of doctors at this period were foreigners. In an attempt to solve the serious situation of shortage in the number of doctors, a medical school was established in the University of Ghana in 1962 after the independence of this country. The shortage of medical doctors was similarly reflected by an insufficient number of teaching staff appointed at the Medical School, and the problem has been serious from the outset. In spite of the maximum effort made by Ghana, it seemed that they had to relay on assistance from other countries to fill the deficiency. A formal request was made also to our country in 1967 through diplomatic institutions.

The University of Ghana Medical School is located at Korle Bu, western boundaries of Accra, capital of Ghana. Accra is also the place where Dr. Hideyo Noguchi died. It is well known that he met a tragic end in Accra in 1928 by the very yellow fever into which he was investigating aetiology with his ultimate efforts. In a process of determining enforcement of medical cooperation in response to the request from Ghana, all the persons concerned would have recalled the death of Dr. Noguchi. That naturally led to request for cooperation to Fukushima Medical College in Fukushima Prefecture, where late Dr. Noguchi was born, and inevitably virology has been selected as the first project in order to complete Dr. Noguchi's unfinished works.

Thus, the author flew to Accra in December 1968 in order to make arrangements for the project. After 10 months of preparatory period including training of two Ghanaians for six months, we arrived in Ghana for the project at the end of September 1969. We landed there as if we were parachute troops, having with us cells of various kinds which can be said as the soul of virology, and minimum quantity of sterilized culture media and pipettes necessary for keeping the cells at least for one month.

On enforcing this project, our objective was "the establishment of a virology laboratory by Ghanaians and for the Ghanaians". There was also the death of Dr. Noguchi as the mental foundation of it. Apart from the mental foundation, the above mentioned objective was literally understood and appealed to them. With the outbreak of yellow fever and wrestling with practical problems as a momentum, it seemed that the objective begun to setle gradually among Ghanaians. It was the most important task for the objective to train Ghanaian counterparts. This problem involving personnel had started with the two Ghanaian technicians, one was specialist in virology and the other as operator of electron microscope, both trained for six months in Japan prior to our arrival to Ghana, followed by the employment of persons from Ghana University and Ministry of Health as the work developed. A thousand emotions crowded on my mind when I was coming back to Japan after the period of twenty two months to find that a total of sixteen Ghanaians including one doctor and two university graduates were receiving training as counterparts in the fields of virology and electron microscopy. At present, these sixteen Ghanaians are expanding the works of virology based on the foundation we built, together with four Japanese specialists from Fukushima Medical College who arrived in July 1971 as the second team.

Research results which are described here are not the objective for this medical cooperation, but are results come out inevitably from the processes of training put into practice as a means to accomplish the objective. Therefore, they are incomplete as research result by itself. In this report, outlines of the results will be described in the order of works we undertook. Parts of the results have already been read[1-3] in scientific conferences and published[1-5] in scientific journals.

I. Yellow fever

Investigations of yellow fever was the unfinished work of the late Dr. Hideyo Noguchi. Therefore this was taken as the starting point of the project. In December 1969 when we had not yet completed unpacking of the machinery from Japan, Dr. Bress, Director of Pasteur Institute, Dakar, visited Accra to talk on the vaccination project by WHO against yellow fever in African countries. Dr. Grant, Epidemiology Division, Ministry of Health invited me to this meeting, we all agreed that it was necessary to make every possible effort in the laboratory towards to the vaccination project, namely, titration of vaccines, evaluation of results and establishment of laboratory diagnosis of yellow fever.

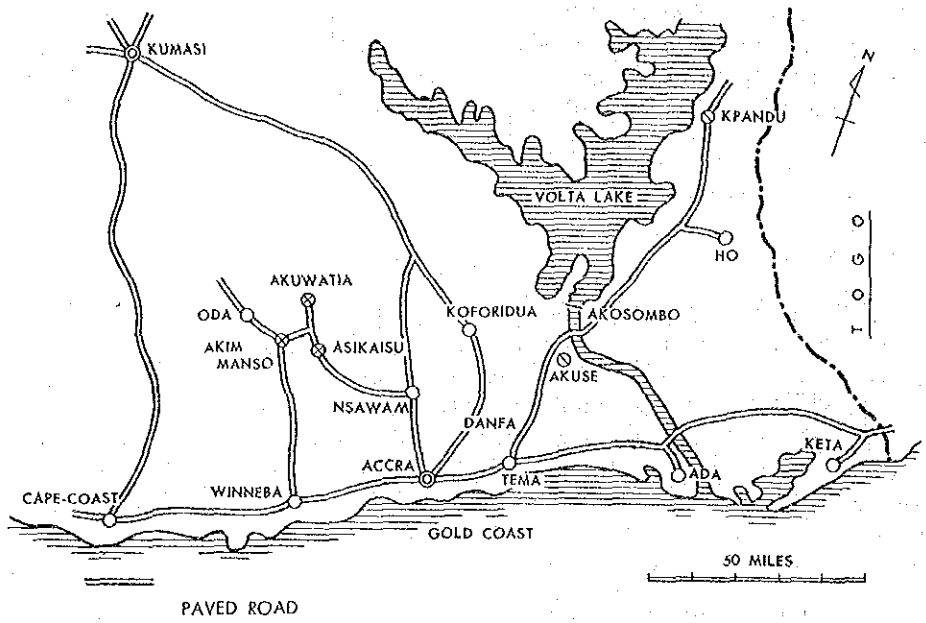
We had some experiences in handling of Japanese encephalitis virus in the laboratory in Japan. Unfortunately we had no experience in yellow fever virus, and we did not have any antigens or sera as standards. I, therefore, requested to Dr. Bress for assistance in these aspects. At the beginning of February 1970, I flew to Dakar on the order of the Ministry of Health of Ghana. I stayed there for one week studying serological diagnosis of yellow fever and isolation methods of viruses, and returned to Accra with the necessary antigens and standard sera. Then, I tried to establish the techniques in the laboratory as soon as possible.

Under these circumstances, at the beginning of April 1970, it was suspected originating from our investigations requested by Dr. Miranda of St. Dominic Hospital, Akwatia that an outbreak of yellow fever had occurred at Akim Manso area near Akwatia. With this as a amomentum, serological study of yellow fever and study of vaccination effect begun to be made throughout Ghana.

1. An outbreak of yellow fever in Akim Manso area

It was from March to May 1970, as mentioned before, when the epidemic occurred at Akim Manso area some 50 miles north west of Accra as shown in Fig. 1. The population of the village was about 3,000 and the village was surrounded by cocoa farms. The farms seemed to me as a jungle since I was only accustomed to Japanese rice field and farms. There was no hospital in Akim Manso area, but St. Dominic Hospital and CAST Hospital belonging to a diamond mine were located at Akwatia some several miles away. Further, Oda General Hospital was located some several miles off to the west. When the

Fig. 1 Southern Part of Ghana



doubt for yellow fever was almost removed, mass vaccination was carried out by the Ministry of Health, and the epidemic ended.

However, during three months from March to May of 1970, about 60 persons died of acute febrile diseases, which were supposed to be yellow fever. Cases which we could examine in laboratory with any sample were from No. 1 to No. 12, as shown in Table 1. Cases from No. 1 to No. 6 were patients hospitalized from March 9 to April 18 at St. Dominic Hospital, who were all sent from Akim Manso area. All patients showed, when hospitalized, severe jaundice, high fever, and vomiting, and all died within 1 to 3 days. Paired sera could be obtained although with three day's interval on cases No. 1 and No. 2 only. HI antibody titer against yellow fever showed more than 1: 10,240 from the onset, and it was impossible to testify increase of antibody titer. However, as for four samples of sera obtained from cases No. 1 and No. 2, IgM fractions with specific activity as III antibody against yellow fever virus were separated by Sephadex G-200, suggesting they were due to acute arbovirus infectious. Especially, the case No. 2 was suspected strongly to be a case of yellow fever, since CF antibody titer was more than 1:256 and neutralizing antibody titer measured in vero cells was also more than 1:1,024.

Case No. 7 was an inpatient at CAST Hospital as acute infectious hepatitis case who was from Akim Manso area. He died three days later. Case No. 8 was a patient found when we were making an epidemiological investigation at Akim Manso area and recovered in a week. Therefore, we could obtain the 2nd serum

Table 1 Laboratory Data for Suspected Cases of Yellow Fever Occurred in Akim Manso, Asikaisu and Akuse, Eastern Region, Ghana, during March to May, 1970

No.	Name	Hospital (a)	Age	Sex	Date	Arb. Group B antibodies (b)			Note
						HI	NT	CF	
1	K. A.	St. Dominic Hospital	30	M	9/3	10,240*	1,024≤	32	
					12/3	20,480*	1,024≤	32	Died
2	O. K.	—do—	50	M	17/3	20,480*	1,024≤	256≤	
					20/3	20,480*	1,024≤	128	Died
3	K. G.	—do—	4	M	6/4	10	<4	<4	Died
4	?	—do—	40	M	13/4	320	32	128	Died
5	S. H.	—do—	?	M	16/4	320	128	128	Died
6	A. K.	—do—	40	F	18/4	20,480	64	NT	Died
7	S. K. N.	CAST. Hosp.	23	M	22/4	20,480	32	128	Died
8	J. K.	Akim Manso	39	F	22/4	10	4	<4	
					28/4	160	1,024	<4	Rec'd
9	K. O.	Akwatia	1.5	M	22/4	20	<4	<4	Died
10	K. A.	—do—	28	M	22/4	80	4	<4	Died
11	O. B.	—do—	5	F	22/4	20	<4	<4	Died
12	O. P.	K'Bu Hosp.	1	M	4/4	20,480	NT	<4	(?)
13	J. O.	K'Bu (Asik.)	11	F	13/5	80	NT	128	Died (c)
14	M. B.	—do—	15	F	13/5	80	NT	4	Died (c)
15	A. B.	Asikaisu	9	F	16/5	640	NT	64	Rec'd (d)
16	B. N.	Akuse	39	F	19/3	160	128	64	
					15/4	640	128	16	Rec'd
17	A. K.	—do—	49	F	3/4	80	64	128	
					16/4	640	128	128	Rec'd
18	D. K.	—do—	18	M	18/3	10	8	128	
					3/4	10	4	64	Rec'd
19	H. T.	—do—	36	F	23/4	<20	<4	<2	
					30/4	80	<4	4	Rec'd
20	P. J.	—do—	13	F	23/5	640	NT	NT	
					6/6	1,200	NT	NT	Rec'd

N.B.: (a) St. Dominic Hospital and CAST Hospital: Located in Akwatia. These patients came from Akim Manso.
(b) Arbovirus Group B antibodies tested by yellow fever virus (FNV) as antigen. NT was done using PS cells by 50% reduction of CPE.
(c) Confirmed by histopathological examination of the post-mortan liver specimens.
(d) Recovered on April, 1970. She lived in the same household with Miss J. O. (case No. 13) and Miss M. B. (case No. 14).
(*) IgM with specific activity was found. Sephadex G-200 column was used for separation of IgM fraction.

from her. At the same time *Aedes aegypti* larvae was found in drinking water in her house. However, a woman in the same house who was healthy when we visited the house on 22nd of April, was dead when we revisited the house a week later. Inspection results on this case No. 8 paired sera showed increases of HI antibody titers from 1:10 to 1:160 and neutralizing antibody titers from 1:4 to 1:10,240. CF antibody titer were less than 1:4. Cases No. 9 to No. 11 on whom we performed blood-letting at Akwatia on 22nd of April had been patients showing

febrile jaundice symptoms, but were all dead when we visited there to get the 2nd sera a week later. Case No. 12 was a patients sent from Akim Manso area to the Korle Bu Hospital at Accra.

As mentioned above, we had rather insufficient clue to make serological diagnosis on infectious cases at Akim Manso area. However, judging collectively from very high mortality rate, certification of high antibody titer, certification of IgM with specific activity and the fact that the prevalence ended finally with enforcement of mass vaccination, we estimated that this prevalence was caused by yellow fever.

On the other hand, two girls (case No. 13 and No. 14) were sent in May from a hamlet called Asikaisu having population of about 300, several miles southeast of Akim Manso to the Korle Bu Hospital at Accra. They were paternal sisters living in the same household. After admitted to the hospital as inpatients, they soon died, and since only acute sera were available, it was impossible to make

Table 2 Details of Unusual Deaths at Asikaisu, Eastern Region, Ghana, April to May, 1970 (By History from Relatives)

No.	Name	House No.	Age	Sex	Approx. Date of Onset	Duration of Illness to Death	Symptoms Reported	Hospitalized or Doctor Seen
1	F. G.	B-85	36	M	Apr. 18	7 days	Vomit, diarrhoea, fever, yellow eyes, scanty urine	Nsawam Hospital, admitted and died after 4 days.
2	K. S.	A-27	31	M	Apr. 18	7 days	Headache, fever, vomit, scanty urine, "green" eyes.	Nsawam Hospital, not admitted. Dr. Sarkwa's clinic—admitted 3 days. Went home and died next day.
3	K. A.	A-25	41	M	May 6	7 days	Fever, vomit, black vomit, scanty urine, constipation, yellow eyes.	Dr. Sarkwa in Nsawam, admitted to his clinic.
4	K. G.	A-23	38	M	May 4	4 days	Fever, black vomit	Asamankese Hospital, not admitted. Nsawam Hospital May 5, admitted and died in 3 days.
5	A. Y.	A-23	29	M	Apr. 15	3 days	Headache, fever, vomit	Asamankese Hospital. Admitted.
6	J. A.	B-95	45	F	Apr. 1	7 days	Vomit, black vomit, stomach pain	Nsawam Hospital admitted 4 days, died at native healer's in Pampanso, near Pakro.
7	A. Y.	B-19	12	F	Mar. 15	5 days	Fever, bloody vomit, yellow eyes, scanty urine	Dr. Sarkwa, died next day. Not admitted.
8	T. Y.	B-19	15	F	Apr. 16	7 days	Bloody vomit, yellow eyes	None

serological diagnosis. However, from pathological examinations of the post-morten liver specimens, they were diagnosed as yellow fever. Immediately I rushed to the site with Dr. Herron, Smallpox Measles Eradication Programmes, Ministry of Health, and performed epidemiological investigation. Case No. 15 was discovered at that time, being a girl living with the Case Nos. 13 and 14. She had just recovered from febrile disease in April. HI antibody titer in her serum was 1:640, and CF antibody was 1:64. Then, it was found out that in this hamlet of Asikaisu there were 13 deaths including two cases sent to the Korle Bu Hospital from March to May 1970. Table 2 shows summary of informations obtained from our visits to the houses of eight patients. It was found through these visits that duration of illness was 3 to 7 days with syndromes which accorded to yellow fever as vomit, fever and yellow eyes. Thus, it strongly suggested that these unusual deaths at Asikaisu were due to yellow fever. Naturally, mass vaccination against yellow fever was effected immediately, and no report of further cases was made.

Now, back to Table 1 and consider five examples from Case No. 16 through Case No. 20. These were cases of febrile diseases occurred from March to May 1970 at Akuse area about 40 miles northeast of Accra. No dead case was reported and all recovered later. For this reason, complete paired sera were obtained enabling serological diagnosis. In all cases, significant increase in HI titer was observed, but as for NT antibody which is said to be more specific, no rise was observed, and fairly high titer in CF was obtained. These data strongly suggested that the epidemic had occurred in this district by some other viruses belonging to group B arboviruses except yellow fever. We had tried virus isolation as possible as we could, but the results were all negative. Materials used were serum or cruor, and inoculation only to BHK21 and vero cells was possible. Unfortunately, inoculation to new born mice was not possible.

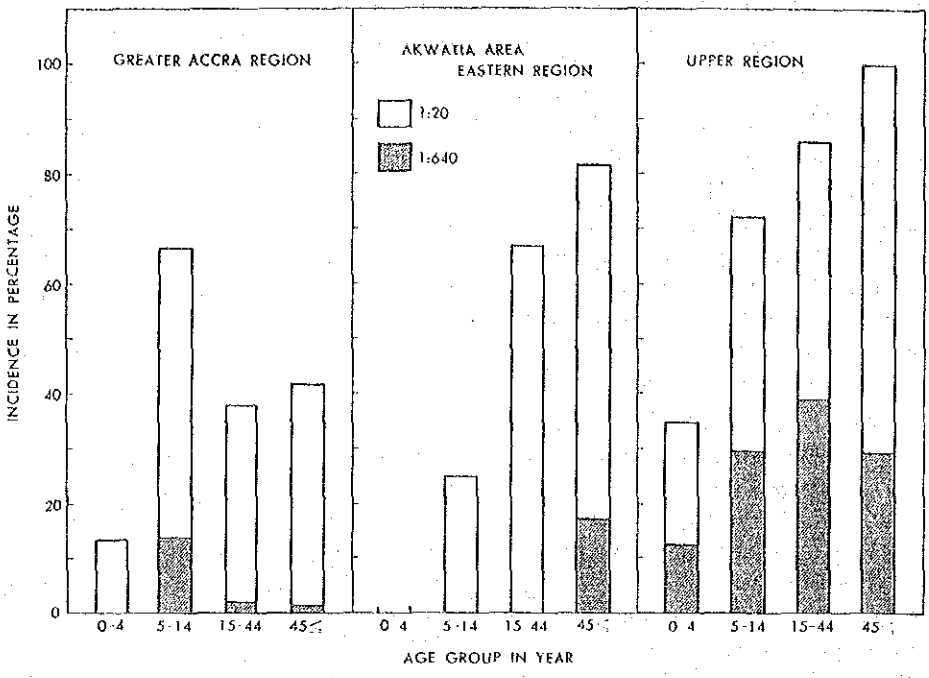
2. Seroepidemiology of Group B arvovirus

With the outbreak of yellow fever in the Akim Manso district, it became interesting to investigate the actual immune status of the population against group B arboviruses throughout Ghana. For this purpose, 224 serum specimens from Accra area, 48 from Akwatia area where the epidemic occurred, and 161 from north Upper Region, totaling 343 serum samples were examined.

These sera were divided into four age groups, namely 0-4, 5-14, 15-44 and over 45 years of age. III tests were performed by using sucrose acetone antigen of yellow fever virus, F.N. strain (apportioned from Dr. Bress of Pasteur Institute in Dakar). The tests were carried out by means of microtiter techniques.

As shown in Fig. 2, no significant difference between these three areas was observed by screening of 1:20, but the Upper Region showed higher incidence as compared with the Great Accra Region and Akwatia area by screening of 1:640. As to age distribution of the HI antibody screened at a level of 1:640, positive incidence in the Upper Region increased as age advanced. Thus, the incidences

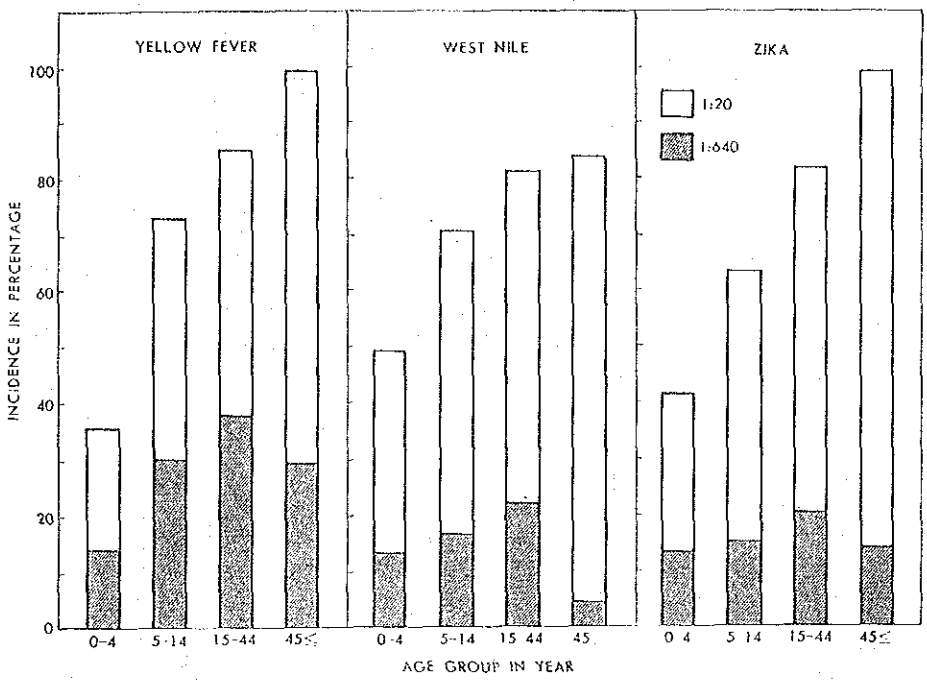
Fig. 2 Haemagglutination Inhibition (HI) Antibodies against Group B Arboviruses (Antigen Used : YF-FNY) in Ghana, 1970



were given as 14.2% for 0-4 years of age, 29.6% for 5-14 years of age, 39.4% for 15-44 years of age, and 29.7% for over 45 years of age. On the other hand, in Akwatia area where the epidemic of yellow fever had occurred, positive incidence at 1:640 was only 18.2% and it was found only in the oldest age group. In the Greater Accra Region, only a few positive persons were found at the screening level of 1:640.

It would be possible that the occurrence of higher antibody titers at the Upper Region reflected an epidemic of yellow wever which reportedly occurred in 1967-68. However, it would be also possible that the antibody was acquired by the mass vaccination which was performed at that time of epidemic. As for 161 serum specimens from the upper region, HI antibody titers were determined against yellow fever, West Nile and Zika viruses all belonging to group B arboviruses and comparison was made among these three. As shown in Fig. 3, no significant differences were observed between the three kinds of antigen by screening of 1:20. Namely, positive rate increased as age grows, and reached to 80-100% for persons over 45 years of age. In a screening of 1:640, the incidence of yellow fever antibody was higher than that of HI antibody against other West Nile and Zika viruses as antigens. This fact shows that the antibodies to group B arboviruses in this district are either due to infection by yellow fever virus or vaccination for yellow fever, and not likely by infection of West Nile and Zika

Fig. 3 Haemagglutination Inhibition (HI) Antibodies against the Types of Group B Arboviruses in the Upper Region, Ghana, 1970

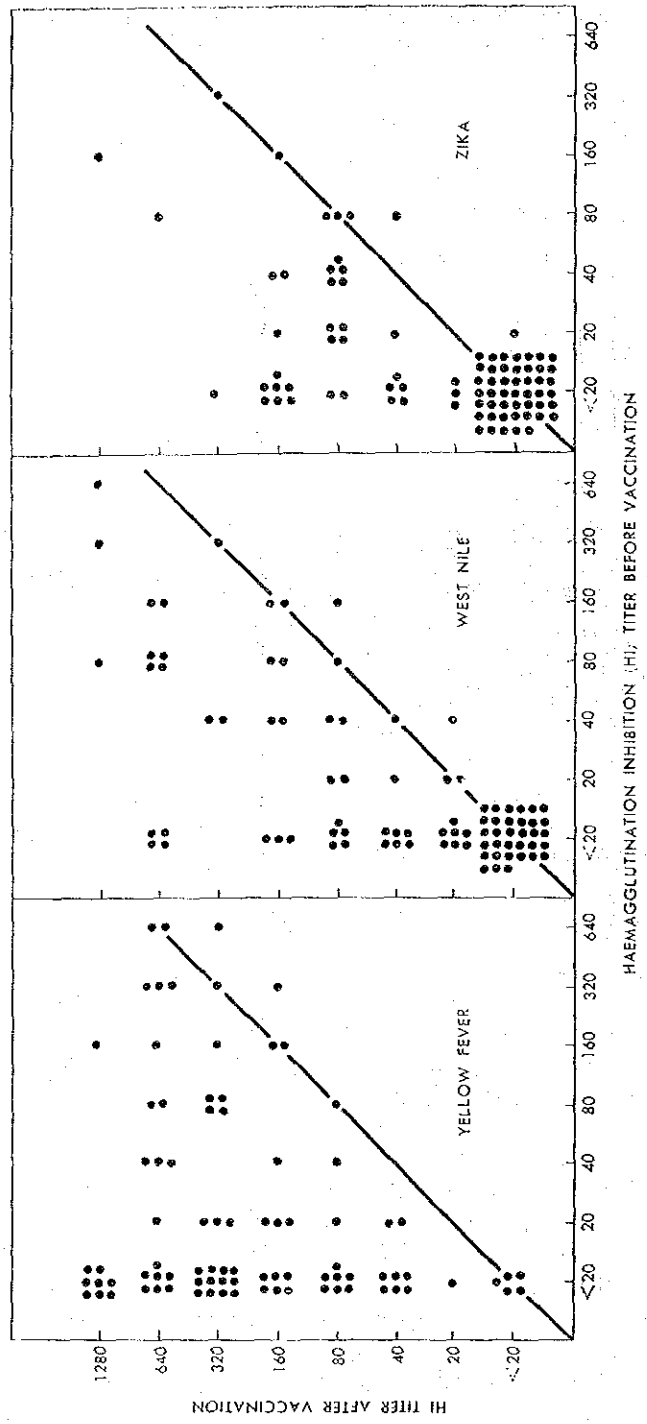


viruses.

3. Immune response of vaccination for yellow fever

With regard to the vaccination of live virus vaccines such as yellow fever vaccination in tropical countries, there are some questions or uncertainly about other hand, we performed vaccination for yellow fever on the staff of the Korle Bu Hospital. On that occasion, we could obtain complete paired sera taken at pre- and post-vaccination stages from 87 persons. On these paired sera HI antibodies were titrated using antigens of homologous yellow fever virus, and heterologuces West Nile and Zika viruses which belong to group B arbovirus. As shown in Fig. 4, antibody response of the vaccination was particularly significant with regard to homologous yellow fever virus. The response was observed on 88% when antibody titer of prevaccination was below 1:20, and almost same degree of response was observed even when the titer was 1:20-1:80. However, in persons whose initial antibody titer was over 1:160, response was so low that only two persons out of 13 showed significant rise in titers. As for West Nile and Zika viruses, the relationship was just reverse; response in case of initial antibody titer being below 1:20 was few and response was observed on persons having some initial antibodies. This fact suggests that persons having some HI antibodies to group B arboviruses are

Fig. 4 Antibody Response of Yellow Fever Vaccination Done for 87 Persons at Korle Bu Teaching Hospital, Accra, Ghana, 1970



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affected by subordinate booster effect after injection of yellow fever vaccine which has same common antigens as a member of group B arboviruses.

In any case, it could be concluded that antibody response of yellow fever vaccination was very good under conditions and environment of the Korle Bu Hospital in Accra. However, it remains true that vaccination at further remote regions under severe conditions should be made with the greatest possible care.

II. Australia antigen

The occurrence of yellow fever epidemic helped our virology laboratory to be established within a short period. Especially, new employment of assistant technicians from the Ministry of Health and establishment of cooperative relations with related hospitals have contributed much for subsequent activities of investigation. Specimens have been sent from throughout Ghana. Until then, at least suspected cases of smallpox and yellow fever had been under obligation to notify to the Ministry of Health immediately, but due to the appearance of our virology laboratory, also specimens have come to be sent to us. Thus, during one year from March 1970, cases which needed serological diagnosis of yellow fever reached to 269 from all over Ghana. Some of them became paired sera, but the majority were single. Except for nine cases which were diagnosed as infection by group B arbovirus at Akim Manso and Akuse areas, no clue was obtained on the remaining 260 cases.

It was the time when Australia antigen discovered by Blumberg in 1964 becoming the object of attention at various parts of the world in its relationship with serum bepatitis. We also paid attention to this, and performed investigations for the Australia antigen by the immunoelectrosyneresis on the 260 cases on which doubts of yellow fever had been negated or no clue had been obtained. As the result showed, Australia antigen was detected on about one-third of the cases. This was really very important in order to make epidemiological investigations of Australia antigen, we gathered energetically blood sera from various parts of the country, and we could investigate for Australia antigen on some 5,000 cases.

1. Australia antigen in patients with jaundice

As mentioned before, out of 269 cases sent to our laboratory as suspected cases of yellow fever or with the intention to negate the doubt, 91 persons (33.7%) were certified by immunoelectrosyneresis (I.E.S.), as having Australia antigen as shown in Table 3. By complement fixation (CF) test, Australia antigen was detected on 145 persons (53.9%).

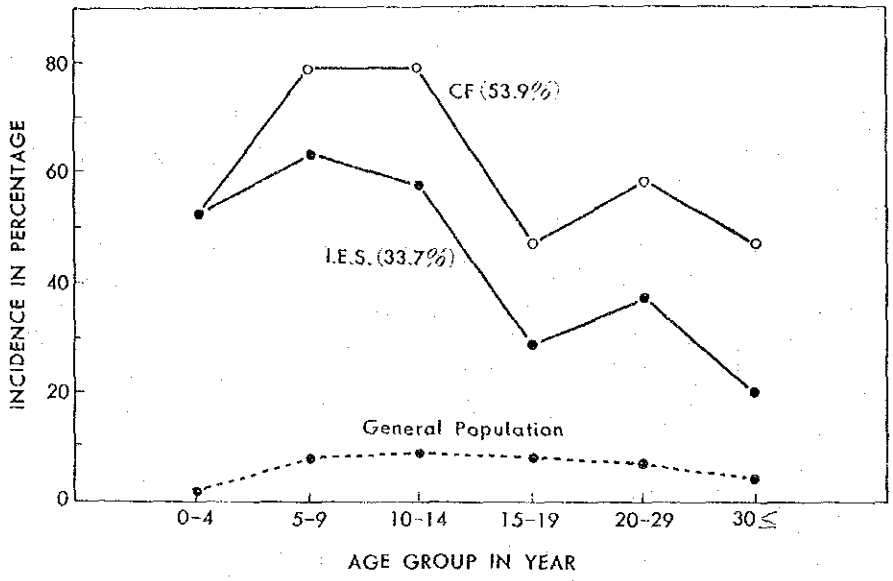
It should be pointed out here that infectious hepatitis had been believed to be very common in Ghana but no such concept had existed on serum hepatitis. Another point to be noted is that many patients are diagnosed clinically as infectious hepatitis, and notification to the Ministry of Health is being made for 5,000 to

Table 3 Australia Antigen and HI Antibodies against Group B Arboviruses among Various Patients with Jaundice Occurred in Ghana 1970-1971

Clinical Diagnosis and Signs	Number Exam'd	Au Antigen				Arbo-B HI Antibodies**	
		I. E. S.		CF			
		Number Positive	%	Number Positive	%	Number Positive**	%
Infectious Hepatitis	188	66	35.1	103	54.7	4	2.1
Jaundice	37	12	32.4	25	67.6	1	2.7
Jaundice with Fever	22	9	40.9	10	45.5	0	0
Yellow Fever	22	4	18.1	7	31.8	4	18.2
Total	269	91	33.7	145	53.9	9	3.4

* Yellow fever SA antigen was used as a common antigen.
** Number of cases in which a definite (4-fold or more) rise in HI titer between 2 suitably spaced sera was demonstrated.

Fig. 5 Age Distribution of Australia Antigen among 269 Patients with Jaundice Occurred in Ghana, 1970-1971



7,00 persons annually. Thus, this disease exists endemically in Ghana, and is very important one. Moreover this disease is occasionally confused with yellow fever. Fig. 5 shows results of investigations made on holding rate of Australia antigen by age of 269 cases with jaundice. Positive rate among children of 14 years of age or younger was very high showing 59% by I.E.S. and 72% by CF test. The rate became lower for persons 15 years of age or older being 21% and 50% respectively. This figure shows that Australia antigen is associated with

cases with jaundice, especially among children under 14 years of age. Electron microsopy was tried on some of them. Namely with regard to 13 cases in which both the I.E.S. and CF tests had showed positive, and 37 cases in which both tests had been found negative, serum samples were separated by ultra ecntrifugation, and the deposit was dealt directly with negative staining and observed by electron microscope. As for positive samples of 13 cases, particles which accorded to Australia antigen of about 20 μ were observed at 100%, and as for the negative samples of 37 cases, similar particles were observed on 26 cases corresponding to 70%. This fact suggests that the positive rate of Australia antigen shown above would become higher if detected by a method with much higher sensitivity.

2. Australia antigen in various group of Ghanaian population

As mentioned before, positive rate by I.E.S. of Australia antigen on 269 cases of jaundiced patients was 33.7%. As for the results on other groups, as shown in Table 4, positive rate of group of 1,092 patients who did not show

Table 4 Prevalence Rate of Australia Antigen in Hospitalized Patients and Healthy Persons in Ghana, 1970-1971

Clinical Group	Number Exam'd	Number Positive	Incidence in %
A. Jaundice Case	269	91	33.7
B. Other Patient	1092	69	6.3
C. Healthy Person	1662	103	6.2
D. Blood Donor	1766	90	5.1
B+C+D (Grand Total)	4520	262	5.8
C+D (Healthy)	3428	193	5.6
B+D (Non-Jaundiced)	2754	172	6.2

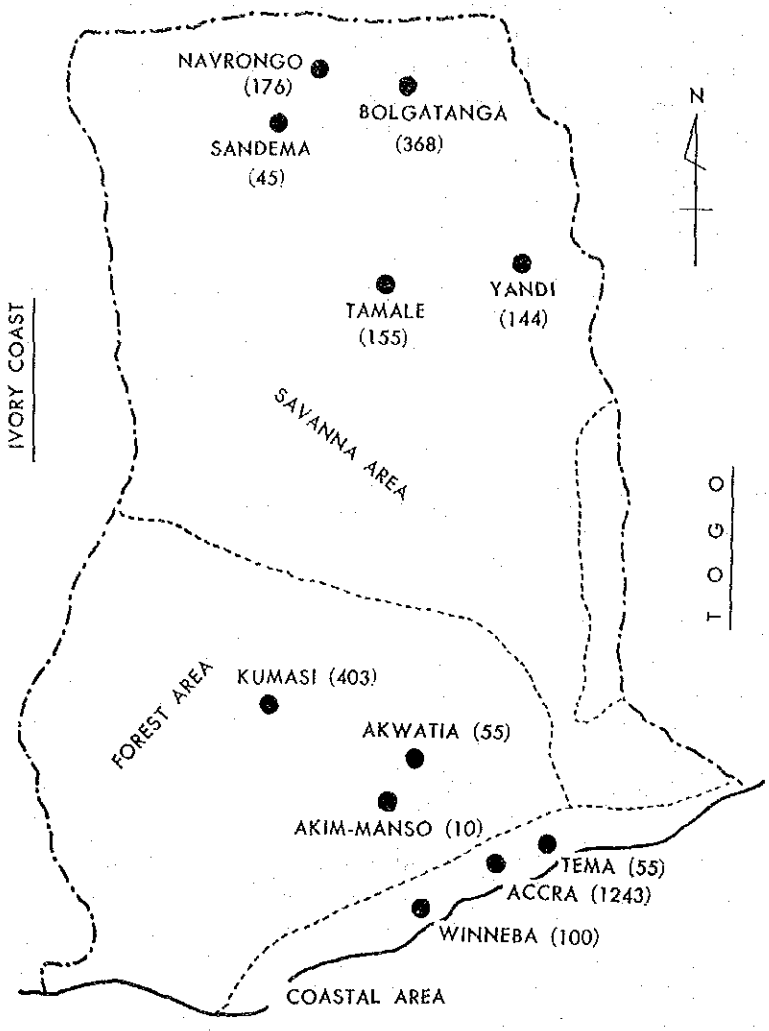
jaundice was 6.3%, that of 1,662 healthy persons from whom blood-letting was made for various purposes was 6.2%, and that of 1,766 blood donors was 5.1%. Blood donors were mainly of 20-30 years of age. and geographically concentrated in Accra area, and were limited to male due to their custom, therefore the group was considered as fairly biased one. On the contrary, age distribution of non-jaundiced patient group and healthy person group were relatively even, and the positive rates were 6.3% and 6.2% respectively. Therefore, by adding these groups to "non-jaundiced person" as an integrated population, we made investigations on distribution status of Australia antigen by age, sex, region and tribe.

Fig. 6 shows names of place and number of sera obtained from 2,754 individuals of this integrated population.

3. Age distribution of Australia antigen

As shown in Fig. 7, positive rate increased gradually as the age develops, namely 1.3% for 0-2 years of age, 2.3% for 3-4 years, and 4.2% for 5-6 years,

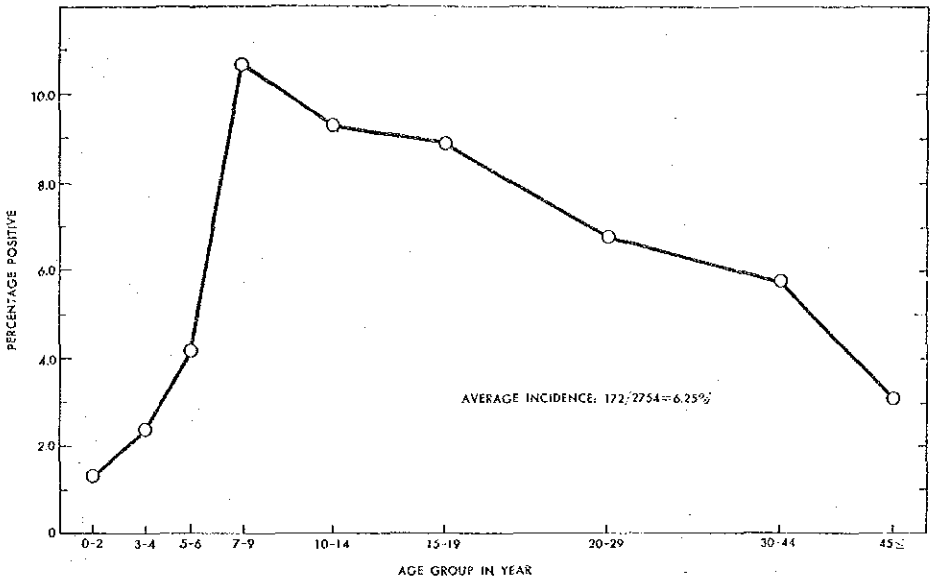
Fig. 6 Place and Number of Sera Collected for Investigation of Australia Antigen in Ghana 1970-1971



and the rate rapidly increased to peak point of 10.7% for 7-9 years of age, and then gradually lowered to the order of 3% for 45 years of age or older. Average rate of the total 2,754 persons was 6.2%.

A total of 150 infants under 2 years of age was investigated, but only two infants had Australia antigen, both being infants of five months. These 150 infants were composed of 20 of 0-2 months, 38 of 3-6 months (out of whom two infants were positive), 25 of 7-12 months, 33 for 1 year, and 34 for 2 years of age.

Fig. 7 Age Distribution of Australia Antigen in Ghana, 1970-1971



4. Difference by tribe and area

As shown in Fig. 6, Ghana can be divided roughly into Coastal area, Forest area and northern Savanna area. By allocating the 2,754 individuals into these three areas as shown in Table 5, further by distinguishing tribes from their names and living area, mentioning cases where more than 10 persons belonged to the same tribe leaving other cases as miscellaneous, and incidence of Australia antigen was investigated by each area.

As for average incidence in these three areas, that of Coastal area was 5.2% which was lower than those of other areas, 7.1% for Forest area and 7.4 for Savanna area. When viewed on relationship with tribes and living area, due to limited number of cases and unequal conditions of selection, there was some fluctuation, but generally we could find a tendency that positive rates of persons in Forest area and Savanna area were higher than that of Coastal area although of the same tribe. Namely, a tendency that difference in living area preceded to that of tribe was observed.

As shown in Table 6, we investigated relationship between clinical diagnoses and appearance frequencies of Australia antigen on 508 patients of groups consisting of more than 10 patients with same diagnosis. Positive rate of Australia antigen in the whole in-patients was 6.1%, which was almost same as for the whole Ghanaians. Out of these patients, clinical groups showing incidence of more than 10% are picked up. Thus, chicken pox of 11% would be mentioned

Table 5 Incidence of Australia Antigen among Various Tribes in Ghana, Speciefid by Area, 1970-1971

Area	Tribe	Number Tested	Number Positive	Percent Positive
Coastal Area	Ga	227	12	5.3
	Fanti	161	3	1.9
	Ewe	90	1	1.1
	Akan	170	12	7.1
	Dagomba	39	3	7.7
	Ga-Adangbe	21	1	4.8
	Other & Unknown	690	41	5.9
	Sub Total	1398	73	5.2
Forest Area	Ga	10	0	0
	Fanti	18	1	5.6
	Ewe	10	1	10.0
	Akan	313	25	8.0
	Dagomba	27	1	3.7
	Other & Unknown	90	5	5.6
	Sub Total	468	33	7.1
Savanna Area	Fanti	56	3	5.4
	Ewe	20	1	5.0
	Akan	114	14	12.3
	Frafra	244	18	7.4
	Grushie	163	11	6.8
	Dagomba	153	9	5.9
	Ga-Adangbe	21	1	4.8
	Komkomba	22	1	4.5
	Other & Unknown	95	8	8.4
	Sub Total	888	66	7.4
Grand Total		2754	172	6.2

first. However, considering that this disease is found chiefly in infant and child ages, this level of positive rate is regarded to be within the normal range. It would be a risky attempt to point out any relationship between Australia antigen and non-specific syndromes such as abdominal pain or swallon. On the other hand, 4 out of 15 adults with anaemia corresponding to 26.4% were positive for Australia antigen, but this fact should be considered with reference to blood transfusion for treatment. Thus, apart from jaundice syndromes already cited, no disease was found which would positively connect with Austrila antigen.

6. The other considerations on Australia antigen

When viewed by sex, positive rate of women was 4.9% which was lower then that of men, 6.7%.

As for antibodies corresponding to Australia antigen, we have investigated on all 2,754 persons to find that, contrary to the case of antigen, the rate was

Table 6 Australia Antigen among Hospitalized Patients in Ghana, 1970-1971

Disease	Clinical Diagnosis	Number Tested	Number Positive	%
Infection diseas	Chicken pox	35	4	11.4
	Leprosy	28	1	3.6
	Malaria	17	0	0
	Tuberculosis (pulmonetary)	35	1	2.9
Enteric disease	Abdorminal pain	37	6	16.2
	Diarrohea	17	0	0
	Other enteric disease	13	0	0
Respiratory disease	Broncho pnemonia	17	1	5.9
	Chest pain	29	2	6.9
	Common cold	42	4	9.5
Tumor	Burkitt tumor	34	1	2.9
Surgical disease	Abscess	13	1	7.7
	Injury (Fracture)	31	2	6.4
	Swollen	19	3	15.8
	Hernia	11	0	0
Gynaecological disease	Abortion	23	0	0
	Delivery	41	0	0
	Pregnancy	11	1	9.1
Other disease	Anaemia (Adult)	15	4	26.4
	Anaemia (Children)	10	0	0
	Anaemia (Sickle cell SS)	20	0	0
	Anaemia (Sickle cell SC, SF)	10	0	0
Total		508	31	6.1

higher for women. Namely, in case of men this was 0.3%, while that for women 1.1%. However, this was about one tenth compared with detection rate of Australia antigen.

III. Enteroviruses

Outbreaks of poliomyelitis in tropical countries are still frequent, and its eradication is the earnest wish of the developing countries. Very effective oral live vaccines have been developed and available for the prevention of poliomyelitis. However, if intestines are occupied with other enteroviruses, "taking" of vaccine will be interfered. We, therefore, had to know the actual status of prevalence of enteroviruses in healthy Ghanaians as a premise for administration of live virus vaccines. We had selected Danfa area as a field which was located some 16 miles north of Accra. In this area, there is a Health Center of the Department of Preventive and Social Medicine under the administration of Dr. Wurapa. With his assistance, we started our studies with an objective of continuing to collect feces from about 30 persons once in every week for a period of

one year. In Danfa area, about 3,000 persons were living in hamlets of about 30 to 100 persons, mainly running agriculture.

1. Prevalence and types of enteroviruses isolated

During one year from May 1970 to April 1971, 253 CPE agents were isolated chiefly in HEp-2 cells from total 1,081 fecal specimens. As shown in Table 7,

Table 7 Isolation of Enteroviruses from Healthy Persons in Danfa Area, Near Accra, Ghana, May 1970-April 1971

Month	Age Group in Year					Total
	0-4	5-9	10-15	16-25	26 & over	
May 1970	3/5 (60.5)	5/9 (55.5)	7/44 (15.9)	6/51 (11.7)	2/10 (20.0)	23/119 (19.3)
June 1970	2/2 (100)	2/12 (16.6)	7/86 (8.1)	0/3 (0)	0/15 (0)	11/118 (9.4)
July 1970	3/3 (100)	7/28 (25.0)	12/53 (22.5)	1/17 (5.9)	0/27 (0)	23/128 (17.9)
Aug. 1970	—	5/12 (41.5)	3/53 (5.7)	1/29 (3.4)	—	9/94 (9.6)
Sept. 1970	6/13 (46.2)	6/16 (37.5)	1/10 (10.0)	0/10 (0)	2/40 (5.0)	15/89 (16.9)
Oct. 1970	3/6 (50.0)	13/65 (20.0)	3/50 (6.0)	—	—	19/121 (15.8)
Nov. 1970	18/34 (53.0)	4/20 (20.0)	2/7 (28.5)	—	—	24/61 (39.4)
Dec. 1970	11/32 (34.4)	9/26 (34.7)	4/11 (36.3)	—	—	24/69 (34.7)
Jan. 1971	23/34 (67.5)	10/19 (52.5)	2/13 (15.4)	—	—	35/66 (53.0)
Feb. 1971	18/28 (64.2)	16/38 (41.2)	2/12 (16.6)	—	—	36/78 (46.0)
Mar. 1971	12/20 (60.0)	4/11 (36.3)	2/8 (25.0)	1/12 (8.3)	3/31 (9.6)	22/82 (26.2)
Apr. 1971	7/9 (77.8)	4/14 (28.5)	0/2 (0)	0/6 (0)	1/25 (4.0)	12/56 (21.4)
Total	106/186 (57.0)	85/270 (31.5)	45/349 (12.8)	9/128 (7.0)	8/148 (5.4)	253/1081 (23.4)

N.B.: Fractions in the table indicate number of viruses isolated/number of persons examined and percentage in brackets.

total efficiency to enterovirus isolation was 23.4%, and it reached up to 57.0% in age group of 0-4 years. The isolation rate decreased as the age develops, but still it was much higher beyond comparison with that of our country. Though some fluctuation in isolation rate was observed by month, considering age composition of samples, no such significant fluctuations seemed to exist during a year.

We had tried to identify these 253 isolated strains of enteroviruses by intersecting horse sera apportioned from WHO, and only 33 strains were identi-

Table 8 **Identification of Enteroviruses Isolated from Healthy Persons in Danfa Area, Near Accra, Ghana, May 1970-April 1971**

Type of Virus	No. Identified
Poliovirus type 1	15
Poliovirus type 2	1
Coxsackievirus B. type 1	1
Coxsackievirus B. type 4	3
Coxsackievirus B. type 5	1
Echovirus type 3	2
Echovirus type 6	1
Echovirus type 11	2
Echovirus type 12	1
Echovirus type 20	1
Echovirus type 24	4
Echovirus type 29	1
Sub Total	33
E-untyped (a)	170
Enterovirus sp. (B)	32
NT-(c)	17
Adenovirus (d)	1
Sub Total	220
Total	253

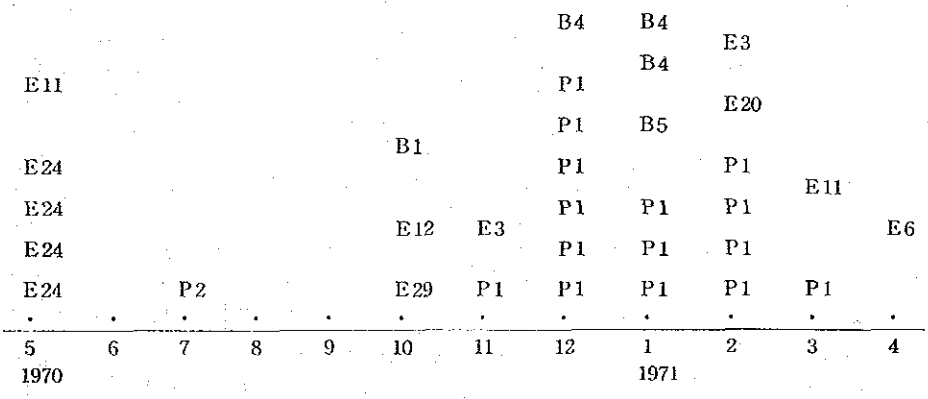
N.B.: (a) E-untyped: Enteroviruses failed in typing using WHO intersecting reference sera, in which the following 42 types of anti-sera were included: Polio—1, 2, 3; Cox. A—7, 9, 16; Cox. B—1, 2, 3, 4, 5, 6; Echo—1, 2, 3, 4, 5, 6, 7, 9, 11 to 33.

(b) Enterovirus sp.: Enterovirus failed in typing using group antisera in which polio 1, 2, 3; Cox. B—1, 2, 3, 4, 5 & 6 antisera were included.

(c) NT: Not tested.

(d) Adenovirus: Excluded to be types 1, 2, 3, 5 & 6.

Fig. 8 Monthly Distribution of Identified Enteroviruses in Danfa Area, Near Accra, Ghana, 1970-1971



E11: Echovirus type 11.
P2 : Poliovirus type 1.
B1 : Coxsackievirus B type 1.

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fied as shown in Table 8. As is seen in Fig. 8, 15 strains of poliovirus type 1 were isolated during the period from November 1970 to April 1971 suggesting that symptomless intection of poliovirus type 1 was prevalent. Also it shows that a prevalence of Echo 24 had occurred in May 1970, and prevalence of small-scale of coxsackie B-4 had also occurred during the period from December 1970 to January 1971. Besides, Polio 2, Echo 3, 6, 11, 12, 20 and 29, Coxsackie B-1 and 5 were isolated sporadically.

2. Occurrence and some characteristics of untyped enteroviruses

Out of the isolated 253 strains, 33 were identified as known virus type. Remaining 220 strains as untyped. Among these untyped enteroviruses, 11 strains were selected as representatives and investigated on their biological, physico-chemical, morphological and immunological characteristics.

As shown in Table 9, these 11 strains were almost same in their cell susceptibility. A marked CPE was observed in HEp-2 and HeLa cells, but as for MK,

Table 9 Biological and Physico-Chemical Natures of Untyped Enteroviruses Isolated from Healthy Ghanaians, 1970-71

Virus No. (Plaque Purified)	Cell Susceptibility (CPE)							HA with Hu-O Cells		Patho. in Suckl. Mice	Physico-Chemical Nature				
	HEp2	HeLa	Vero	HEF 6-2	HEF 3-4	HELi 1-5	MK	37°C	4°C		IUDR Test	Ether Stab.	Acid Stab.	Cation Stab.	Thermo Stab.
No. 2	++	++	−	−	+	+	−	−	−	No	RNA	S	S	S	L
No. 3	++	++	−	−	+	−	−	−	−	No	RNA	S	S	S	L
No. 89	++	++	−	−	+	+	−	−	−	No	RNA	S	S	S	L
No. 110	++	++	−	−	+	+	−	−	−	No	RNA	S	S	S	L
No. 150	++	++	N	N	N	N	−	−	−	Yes	N	S	S	S	L
No. 915	++	++	−	−	+	++	−	−	−	No	RNA	S	S	S	L
No. 931	++	++	−	+	+	+	+	−	−	No	RNA	S	S	S	L
No. 1116	++	++	−	−	−	−	−	−	−	No	RNA	S	S	S	L
No. 1118	++	++	−	−	+	+	−	−	−	No	RNA	S	S	S	L
No. 1285	++	++	−	+	+	+	−	−	−	No	RNA	S	S	S	L
No. 1408	++	++	−	+	+	+	−	−	−	No	RNA	S	S	S	L

N.B.: S: Stable, L: Labile, N: Not Tested.

only one strain No. 931 showed CPE. No CPE was observed in vero cells. In case of diploid cells from human fetus, some of them were susceptible and others were not susceptible depending on virus strains and cell lines tested. In animal inoculation experiments, only one strain No. 150 was pathogenic in suckling mice. Haemagglutination tests with human O red cells were negative either at 37°C or at 4°C. All strains tested were proved to be of RNA type by IUDR tests. They were ether tolerant, acid tolerant, positive in cation stabilization test, labile in thermo stabilization, hexagonal form of about 23 μ in diameter under electron microscope. All of which accord general characteristics of enterovirus.

Rabbit immune sera were prepared against the representative 11 strains of untyped exteroviruses. These strains were able to be devided into 4 main groups, namely group Nos. 2, 915, 1118, and others by cross neutralization tests using these rabbit immune sera. Cross neutralization tests were further performed between 202 strains of untyped enteroviruses and representative rabit

Table 10 Monthly Distribution of Enterovirus Isolations, Untyped and Typed, from Healthy Persons in Danfa Area, Near Accra, Ghana, during a Period between May 1970 and April 1971

Month 1970 to 1971	Number of Viruses Isolated	Number of Untyped Isolated				Type and Number** of Viruses Identified	No. of Viruses Not Tested
		Group No. 2	Group No. 915	Group N. 1118	Other		
Mar. 1970	23	9	3	1	5	E11(1), E24(4)	0
June	11	2	0	1	5		3
July	23	5	2	0	13	P2(1)	2
Aug.	11*	3	2	1	5		0
Sept.	16*	5	4	0	6		1
Oct.	20*	7	2	0	4	E12(1), C B 1 (1), E29(1)	3
Nov.	24	8	2	6	5	P1(1), E3(1)	1
Dec.	24	3	1	2	7	P1(6), C B4(1) A(1)	3
Jan. 1971	35	6	0	0	19	P1(3), C B5(1), C B4(2)	4
Feb.	36	4	2	8	14	P1(4), E3(1), E20(1)	2
Mar.	22	3	1	1	14	E11(1), P1(1)	1
Apr.	12	1	0	0	9	E6(1)	1
Total	257	56	19	20	107	34	21

* Four isolates, 2 in August and each 1 in September and October, 1970 were mixed culture in which 2 viruses belonged to the groups No. 2 and No. 915 were involved.

** E11: Echovirus type 11. P2: Poliovirus type 2. CB1: Coxsackievirus B type 1. A: Adenovirus. Figure in brackets indicates the number of viruses identified.

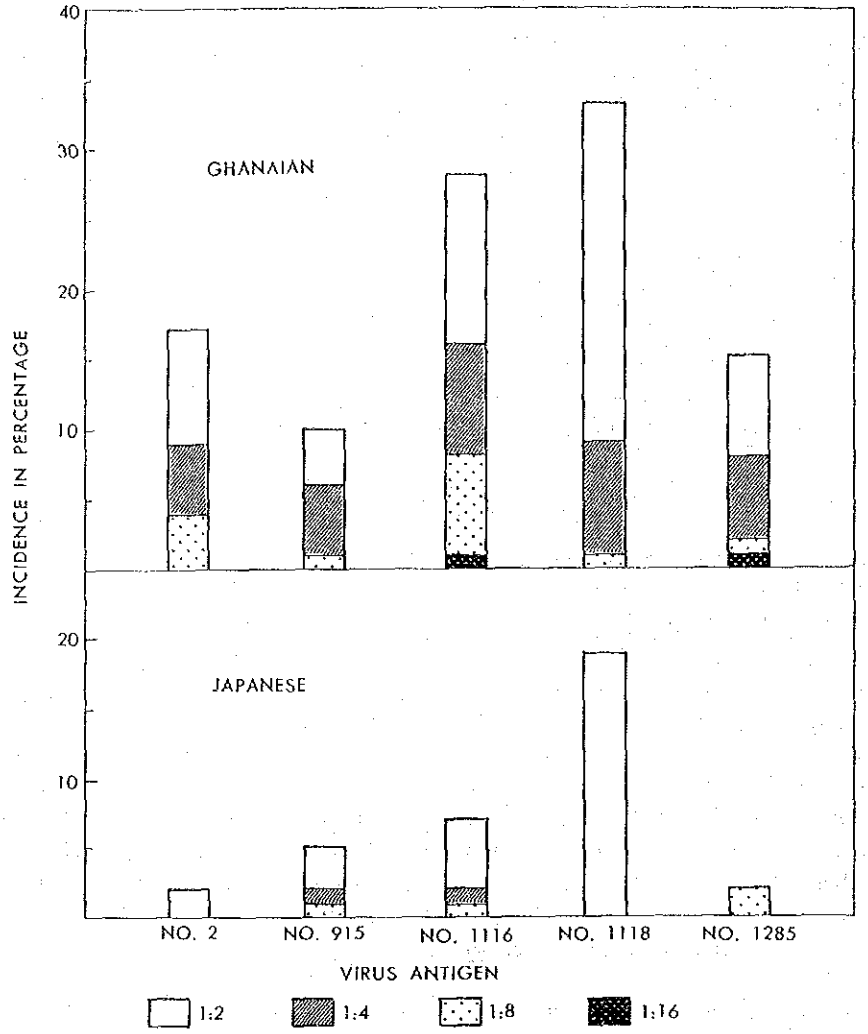
immune sera. Thus, as shown in Table 10, 56 strains were proved to belong to group No. 2, 19 to group No. 915 and 20 to group No. 1118, totaling 95 strains grouped. A consideration was taken on monthly distribution of enterovirus isolations belonged to these groups. No significant difference in isolation efficiency was observed from month to month suggesting an endemic status of these groups of untyped enteroviruses in the healthy persons in the area investigated.

3. Relationship to human being

These untyped viruses were isolated without doubt from feces of healthy Ghanaians, and in this respect, were enteroviruses of human being. However, it was quite unknown on relationship to human being. In order to ascertain this point, blood sera of 100 Ghanaians and 100 Japanese were selected at random, and neutralization antibodies against the representative 5 strains were determined. Antibody holding rate was generally low, as shown in Fig. 9. Especially, in case of Japanese, it was significantly lower than that of Ghanaians, but not zero.

Similar six untyped enteroviruses were isolated by measles project, which will be described later, and complete paired sera were obtained in all cases. Thus, neutralization antibody titers were determined against these isolates but all were below 1:4.

Fig. 9 Occurrence of Neutralizing (NT) Antibodies against Untyped Isolates in Ghana, 1970-1971 and Japan, 1966



Further consideration should be paid on relationship between these untyped enteroviruses and "Apollo 11 Disease" which originated in Ghana in 1969 causing pandemic, and later attacked Japan in 1972 under the name of new type of acute haemorrhagic conjunctivitis (AHC). On the epidemic of AHC in Japan, a virus tentatively named as AHC virus was isolated by Dr. R. Kono of National Institute for Health of our country. After making cross neutralization tests between representative strains of untyped enterovirus from Ghana and the AHC virus, it was proved that the both had no relation at all.

This study of enterovirus was made as a fundamental experiment as a

premise for general administration of oral live virus vaccine, and enteroviruses were isolated very efficiently. Moreover, many of these isolates did not accord to known types of enteroviruses and were considered to be new types of virus. Whether these will interfere with live polio vaccine or not may only be determined by actual trial under carefully controlled conditions. Further, relations of these untyped viruses and human being and with nature should be a problem to be pursued in future.

IV. Measles

Measles in tropical Africa is a very important disease. Many of infants' deaths are considered to be caused by measles. During the period from 1969 to 1971 in which we stayed there, the Smallpox-Measles Eradication Project by the United States happened to be in progress. In spite of the project, reports of measles patients remained at high level. Therefore, we felt keenly the necessity to know actual situations of measles based on laboratory diagnosis. We wrestled with this problem after consulting with Dr. Herron of Smallpox-Measles Eradication Project of the U.S., and Dr. Ofosu-Armah in pediatrics.

Our project was to make serological and virological investigations on two clinical groups, namely measles and non-measles, which were carefully grouped by two specialists, a nurse and a paediatrician. Thus, 36 cases were grouped as measles, and 26 cases were for non-measles group.

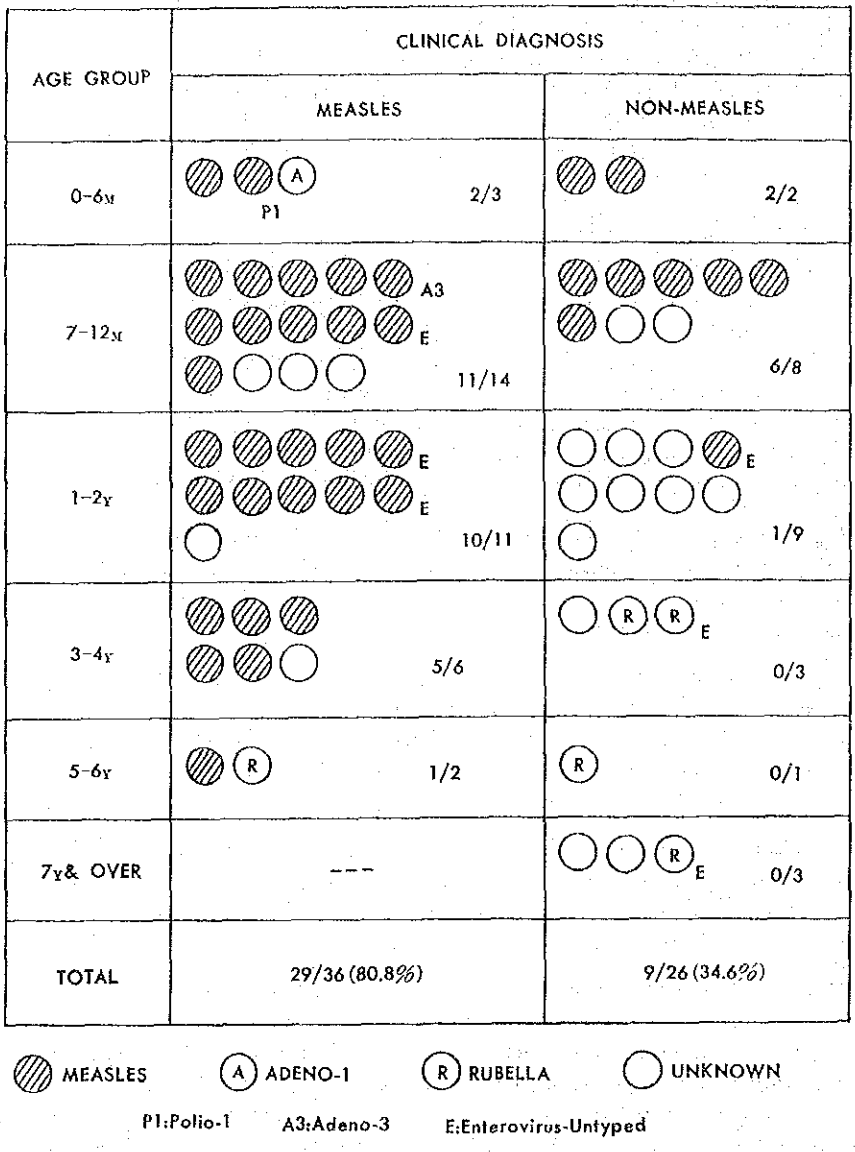
Out of 36 patients diagnosed clinically as measles, 29 (80.8%) were confirmed, as shown in Fig. 10, as measles serologically. Both the hemagglutination inhibition antibodies and neutralizing antibodies were at high level. One case by adenovirus type 1 and another case by rubellavirus were included. No clue was obtained on 5 (13.6%) patients.

One case was found in this group in which poliovirus type 1 was isolated and shown responses of antibodies against both measles and polio type 1. There was one case in which adenovirus type 3 was isolated simultaneously, and three cases were associated with untyped enterovirus.

Out of 26 patients diagnosed clinically as non-measles, 9 cases (34.6%) were confirmed serologically as measles. Though 4 cases (15.4%) of rubella were found, no clue was obtained on the remaining 50%. There was one case in which antibodies against measles increased and untyped enteroviruses were isolated but without increase of rubella antibodies. Untyped enteroviruses were isolated in 6 cases from measles and non-measles groups, but in no case increase of neutralizing antibody titer against isolated viruses was observed.

These results suggest that in Accra there exists measles confirmed by laboratory diagnosis. In spite of enormous efforts by the Smallpox-Measles Eradication Project team, it seems to be difficult to eradicate measles. Most of the clinical measles were also confirmed as measles in laboratory, but some

Fig. 10 Clinical and Laboratory Diagnosis of Measles and Resemble Eruptions in Accra, Ghana, 1971



of them, although small in number, were not genuine measles. These confusing cases were found mostly in infants of less than one year old. On the other hand, it is also important that the existence of rubella was confirmed through this project.

V. Suspected case of smallpox

Smallpox is a very important viral disease for human beings. Ghana had been until recently, like other tropical Africa, one of the prevailing area of smallpox. The Smallpox-Measles Eradication Project of the United States established since 1967 started its activities in 19 countries in Central and West Africa including Ghana, and no genuine smallpox case has been reported in Ghana since 1968. However, suspected cases of smallpox occurred frequently there. During the period from January 1970 to March 1971, 15 such cases were reported. Although no case of genuine smallpox was certified, it is necessary to make laboratory diagnosis on these suspected cases promptly together with clinical and epidemiological investigations.

For this purpose, there are many methods conceivable such as detection of antigen by precipitation reaction in gel, isolation of viruses in tissue cultures or embryonated chicken eggs, diagnosis including direct observation of viral particles by electron microscope and serological tests. These methods have been established in the laboratory gradually. Some of the materials were sent

Table 11 Summary of Clinical and Laboratory Investigations in Suspected Smallpox Cases in Ghana—1st January, 1970 through March, 1971

CASE NO.	AGE	SEX	CLINICAL DIAGNOSIS	LABORATORY SPECIMENS AND RESULTS: Specimen Type	Date of Receipt	E. M.	Agar Gel	Tissue Culture	Chick (CAM) Culture	FINAL DIAGNOSIS
1	32	M	Probable Smallpox	Pust. Fluid	15/1/70	Neg.	Neg.	Neg.	Neg.	Severe
				" "	18/1/70	Neg.	Neg.	Neg.	Neg.	Chickenpox
				Scabs	18/1/70	Neg.	Neg.	VACCINIA	VACCINIA	with active
				"	30/1/70	—	Neg.	Neg.	—	Smallpox
				Serum No. 1	15/1/70	—	—	Neg.	Neg.	Vaccination
				" No. 2	18/1/70	—	—	Neg.	Neg.	
				" No. 3	30/1/70	—	—	—	—	
2	45	M	Chickenpox (Poss. SP)	Pust. Fluid	16/1/70	HERPES	Neg.	Neg.	Neg.	Chickenpox
				Scabs	16/1/70	HERPES	Neg.	Neg.	Neg.	
3	14	M	Chickenpox	Pust. Fluid	18/2/70	HERPES	Neg.	Neg.	Neg.	Severe
				Scabs	18/2/70	HERPES	Neg.	Neg.	Neg.	Chickenpox
				"	5/3/70	—	Neg.	Neg.	—	
				Throat Swab	19/2/70	—	—	Neg.	Neg.	
				Rectal Swab	19/2/70	—	—	Neg.	Neg.	
				Serum No. 1	18/2/70	—	—	Neg.	—	
4	35	M	Chickenpox	Scabs	25/2/70	—	Neg.	Neg.	—	Chickenpox with facial scars
5	NA	NA	Chickenpox	Scabs	5/3/70	—	Neg.	Neg.	—	Chickenpox
6	NA	NA	Chickenpox	Pust. Fluid	5/3/70	HERPES	Neg.	Neg.	—	Chickenpox
				Scabs	5/3/70	HERPES	Neg.	Neg.	—	
7	6mo.	F	Chickenpox	Scabs	13/3/70	HERPES	Neg.	Neg.	—	Chickenpox
8	40	M	Chickenpox (Poss. SP)	Pust. Fluid	7/4/70	HERPES	Neg.	Neg.	—	Chickenpox
				Scabs	7/4/70	HERPES	Neg.	Neg.	—	
9	15	M	Chickenpox	Scabs	9/4/70	—	Neg.	Neg.	—	Severe Chickenpox
10	27	M	Chickenpox	Pust. Fluid	7/8/70	Neg.	Neg.	Neg.	—	Severe
				Serm No. 1	7/8/70	—	—	Neg.	—	Chickenpox
11	31	M	Chickenpox (Poss. SP)	Scabs	15/9/70	HERPES	Neg.	Neg.	—	Chickenpox
12	54	M	NA	Scabs	18/11/70	—	Neg.	Neg.	—	Not Smallpox
13	16	M	Chickenpox (Poss. SP)	Pust. Fluid	21/11/70	HERPES	Neg.	Neg.	—	Chickenpox
				Scabs	21/11/70	HERPES	Neg.	Neg.	—	
14	55	M	Chickenpox (Poss. SP)	Vesic. Fluid	24/2/71	HERPES	Neg.	Neg.	Neg.	Severe
				Scabs	10-3/71	HERPES	Neg.	Neg.	Neg.	Chickenpox
				Serum No. 1	10/3/71	—	—	Neg.	Neg.	
15	44	F	Unexplained Death with rash on thighs	Skine lesion (post-mort.)	22/3/71	HERPES	Neg.	Neg.	—	Herpes simplex or zoster; terminal hematologic illness

Abbreviations Used: Neg.=Negative; —=Test not performed; NA=Information not available; SP=Smallpox; Pust.=Pustular; Vesic.=Vesicular; mo=Months.

to the Communicable Disease Center (CDC) in the United States for comparison of results.

Table 11 summarizes clinical, epidemiological and laboratory data on suspected cases occurred during the period from January 1970 to March 1971. No genuine smallpox case was found and all were cases suggesting strongly to be chicken pox. Case No. 1 was a case which seemed heavy rash due to chicken pox, and vaccination for smallpox was effected when visited clinic, and took over by vaccinia virus. Vaccinia virus was isolated from scabs of this case.

VI. Apollo 11 disease (acute haemorrhagic conjunctivitis: AHC)

It was a new type epidemic haemorrhagic conjunctivitis called "Apollo 11 Disease" that we had to face when we arrived in Accra on 30th of September 1969.

Due to severely restricted conditions of laboratory, samples were carefully selected, after consulting with Dr. Chatterjee of eye clinic. The eye swabs from acute 15 cases, whose symptoms appeared on the day or the previous day, were taken at bed side and were immediately inoculated to tissue culture cells such as HeLa, Vero and HEp-2. No CPE had, however, appeared in any of the tissue culture cells inoculated even after three blind passages, giving a result of negative virus isolation. Long time after that, as we could obtain kidney of fetus by caesarean operation, we prepared human embryo kidney tissue cultures. Reinoculation of the specimens which had been kept at –20°C was performed using these kidney cells, but the virus isolation was also negative.

Measurements were made on CF antibody titers against adeno- and herpersviruses on paired sera obtained completely from these 15 patients, but no increase of antibody titers was observed. Hence, we had to conclude as negative from the virological point of view. These data were introduced in papers of Dr. Chatterjee.

"Apollo 11 Disease" later landed on Japan in August 1971 via African countries including Nigeria, India, Southeast Asian countries and Taiwan, and prevailed in Japan as north as Hokkaido. Dr. Reisaku Kono and other researchers of the National Institute for Health isolated virus which seemed to be as causal virus. As I described earlier, since the virus resembled much to the untyped enteroviruses isolated from Ghanaians at Danfa, we had compared both of them serologically and found that they did not cross each other.

We have investigated neutralizing antibodies of sera from 145 Ghanaians against this AHC virus isolated by Dr. Kono and others. These sera were taken during the period from 1970 to 1971 which was after the prevalence of the Apollo 11 Disease. As shown in Table 12, 65% was positive in screening of 1:4. No significant difference between age was admitted. However, in screening of 1:64, 40% were positive for young age group of 0-10 years of age, and the percentage was lower for older person.

Table 12 Age Distribution of NT Antibodies against AHC Virus in Ghana, 1970-1971

Age Group	Number Exam'd	Number Positive at		
		1:4 ≦	1:16 ≦	1:64 ≦
0-10	25	18(72)*	13(52)	10(40)
11-20	35	21(61)	11(31)	5(14)
21-30	31	21(68)	13(42)	6(19)
31 ≦	34	19(56)	9(27)	2(6)
Sub Total	125	79(63)	46(37)	23(18)
Unknown	20	15(75)	5(25)	1(5)
Grand Total	145	94(65)	51(35)	24(17)

* Figure in brackets indicates percentage.

It is very interesting that age distribution of this serum antibody and patients caught actually by this Apollo 11 Disease were mostly adult, and no case of infants was reported. These data also show that antibodies against AHC virus were widely distributed among Ghanaians. On the other hand, Prof. Ishii of Hokkaido University isolated AHC virus by HeLa cell from Japanese cases, and it is said that susceptibility of HeLa cells to this virus depends on cell line used. HeLa cells we took to Ghana were those which were apportioned from this laboratory, and it was confirmed recently that AHC virus which we received from Dr. R. Kono did not show CPE in our HeLa cells. Thus, we could understand why CPE agent could not be isolated with HeLa cells we used in Ghana. It is strange that Japan and Ghana are related in quite unexpected phase.

VII. The other viral diseases

Measurements of antibody value against various kinds of viruses were made on 86 Ghanaians sera which were collected during my first visit to Ghana in December 1968. These were, although limited in number, important as sero-epidemiological data on viral diseases of Ghanaians at that time, and were not referred to in previous articles. I, therefore, will introduce its outline here.

Serum specimens were those which were sent to the clinical laboratories of the Korle Bu Hospital for Kahn and Widal tests. All the measurements were made based on micro-titer techniques. Measurements of neutralizing antibodies were made, as shown in Table 13, on each type of poliovirus, coxsackievirus type B-5, adenovirus type 3 and measles virus. Neutralizing antibodies for each type of polioviruses were detected at high percentage, as expected. Moreover, they reached to the maximum level already in child age. On the contrary, neutralizing antibodies against coxsackievirus B-5 were generally low, and found much in adult age rather than in infant age, and only one person had antibody titer of 1:64. Antibodies against adenovirus type 3 were found at fairly high rate. Almost 100% had antibodies against measles with high titer which shows high percentage of infiltration of measles in that country.

Table 13 Antibodies against Various Viruses in Ghana, 1968

Type of Test and Virus Antigen	Number Exam'd	Number Positive At an Antibody Titer of		
Numeralization		1 : 4≦	1 : 16≦	1 : 64≧
Polio type 1	86	79 (92)*	55 (64)	33 (38)
Polio type 2	86	76 (88)	43 (50)	17 (20)
Polio type 3	86	80 (93)	43 (50)	15 (17)
Coxsackie B-5	80	25 (31)	2 (3)	1 (1)
Adeno type 3	72	57 (79)	38 (53)	17 (24)
Measles	86	85 (99)	80 (93)	53 (62)
Complement Fixation		1 : 4≦	1 : 8≦	1 : 16≦
Adeno	77	19 (25)	7 (9)	5 (7)
Herpes	77	33 (39)	12 (16)	3 (4)
Haemagglutination Inhibition		4 : 8≦	1 : 32≦	1 : 128≦
Influenza				
A2/Murakami/64	70	57 (82)	43 (61)	27 (39)
A2/Kumamoto/67	70	27 (39)	8 (14)	3 (4)
A2/Aichi/68	70	21 (30)	3 (4)	1 (1)
B/Tokyo/67	70	7 (10)	3 (4)	1 (1)
Rubella	60	37 (63)	29 (48)	13 (20)
Japanese		1 : 10≦	1 : 40≦	1 : 320≦
Encephalitis	62	18 (29)	14 (23)	8 (13)

* Figure in brackets indicates percentage.

CF test was performed with adeno and herpes viruses, and the incidences at 1:4 were 25% and 39% respectively.

HI test was performed on various types of influenza, rubella and Japanese encephalitis viruses. As for influenza virus, antibodies against A_2/Murakami/64 were at high level. A_2/Aichi/68 is A_2 influenza virus of so-called "Hongkong" type and it is very interesting that antibodies with high value against the virus were detected although small in number in Accra suggesting its infection in December 1968 when antibodies to this virus were not yet detected in Fukushima that locates in northern Japan. As for influenza B, antibodies were lower than those of each type of A.

It was found that there existed antibody against rubella virus. It seemed that antibodies detected against Japanese encephalitis virus would be of common antigen with arbovirus group B, especially with yellow fever. Moreover, both viruses resemble each other in their antigen structures. When viewed from epidemiological point it is very interesting that although *aedis aegypti* grows in inflicted zone by Japanese encephalitis—Japan, East Asia, Southeast Asia and Pacific area—but no case of yellow fever has occurred, while no case of Japanese encephalitis has occurred in yellow fever prevailing area.

Acknowledgements

Publication of this report has been planned by the International Medical Foundation of Japan, and I would appreciate for giving me the opportunity of its publication. I wish to extend my sincere appreciation to Overseas Technical Cooperation Agency (OTCA), Fukushima Medical College, Japanese Embassy in Ghana, Ghana University, Ministry of Health of Ghana, for their kind assistance both officially and privately extended to me through the medical cooperation with Ghana.

This medical cooperation is the fruit of good intentions and efforts of all kinds of people—not only Japanese and Ghanaians, but also all the people in the world, namely African, American, Englishman, Canadian, Korean, Filipino, Chinese, professors, doctors, engineers, nurses, office clerks, merchants and operators and others. Taking this opportunity I wish to express my deep gratitude to all of these well-known and unknown people.

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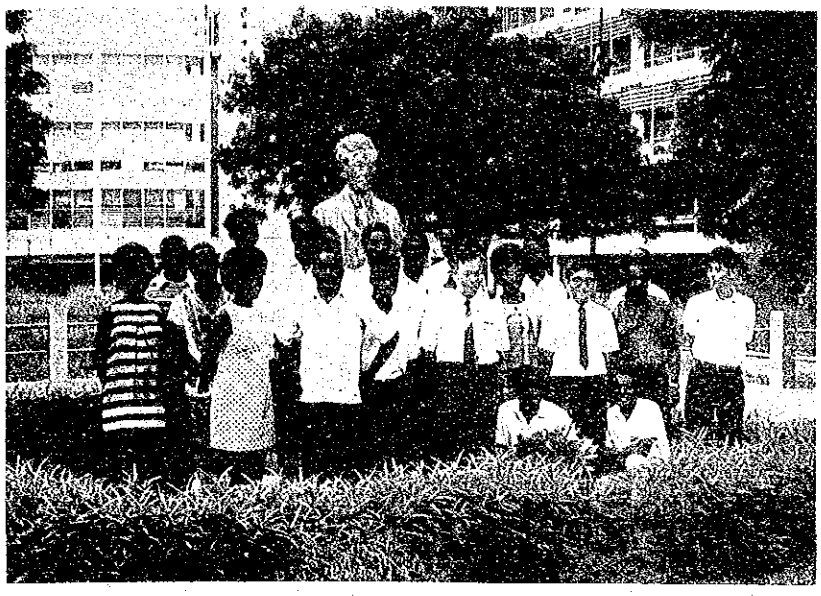

Japanese specialists and the Ghanaian counterparts in front of the Bust of Dr. Hideyo Noguchi in the compound of the Korle Bu Teaching Hospital in Accra, the Surgical Ward seen in the background.

Frm left Prime Minister Busia, the author giving a briefing, Health Minister Ampaw and Professor C. O. Easmon, the Dean (the titles are of the time picture taken).

PREMIER BUSIA VISITS KORLE BU

● Premier K. A. Busia has visited the Medical School at Korle Bu, Accra, to acquaint himself with developments taking place there. He is pictured at the Glass Ware Preparation Room listening to a point of interest by Professor Kazumori Minami, a virologist of the school. The Premier was accompanied by Health Minister Ampaw.

Newspaper Cuttings from the "Weekly Spectator" of June 27, 1970. The cleaning room of the Virology Department Medical College of Ghana University.

Weekly Spectator

3 SATURDAY, JUNE 27, 1970

「日本医事新報」別刷（第二五六八号）
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[I-10]

アポロ一一病の病因について

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MINAMI, K. and KONNO, K.:
POSSIBLE ETHIOLOGY OF "APOLLO 11 DISEASE"
JAPAN MEDICAL J. 2568, 32 - 34, 1973.

Sixty five sera collected before the epidemic of Apollo 11 Disease and 145 collected after the epidemic in Ghana were examined for neutraliging (NT) antibody against AHC virus which was isolated as a causative agent of the epidemic of acute haemorrhagic conjunctivitis occurred in Japan 1971.

Very high incidence of NT antibody was found in sera collected after the epidemic, while it was very low before the epidemic, suggesting that AHC virus isolated in Japan could also be quite possible to have played as a causative agent of Apollo 11 disease in Ghana.

MINAMI, K., and KONNO, K.:
Possible ethiology of Apollo 11 disease,
Japan Med. J. 2568, 32-34, 1973.

(昭 48. 7. 14 日——No. 2568) 32

アポロ一一病の病因について

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はじめに

一九七一年八月頃から数カ月にわたり、かつて見られなかつた型の急性出血性結膜炎（Acute Haemorrhagic Conjunctivitis：AHC）が日本全土を一過性に席巻した[1,2]。その病因ウイルスとして、国立予研の甲野博士らはピコルナウイルス群に属するが新型の可能性の強いAHCウイルスを分離した[3]。

日本における流行の約二年前の一九六九年六月に、これと臨床的、疫学的にきわめて酷似した出血性結膜炎の爆発的流行が、西アフリカのガーナで発生した[4,5]。ちょうどその頃、アポロ一一号が人類最初の月着陸に成功し、ガーナでは誰いうともなく、この原因不明の眼疾患を「アポロ一一病」または「アポロ眼」と呼ぶようになつた。その後、この流行はナイジェリア[6,7]、セネガール等の西アフリカ諸国にひろがり、やがて中近東、インド亜大陸、東南アジア等の広範な地域に拡大し、約三年間にわたるパンデミーをひきおこした[1,3]。

著者らは、たまたま一九六九年一〇月はじめ、海外技術協力事業団（OTCA）の要請により、ガーナにおけるウイルス学のプロジェクト実施のため現地に着任した。当時、ガーナではやや下火になつたとはいえ、「アポロ眼」の流行の最中であり、私どもは急遽本疾患のウイルス学的検索を行なう破目となつた。しかしながら、ウイルス分離は不成功に終り、血清学的にも既知のアデノおよびヘルペスウイルスの感染が否定され、結局、原因ウイルスを結論するまでには至らなかつた[4,5]。

甲野博士らによつて日本の流行例から分離されたAHCウイルスが、原因不明に終つたガーナの「アポロ一一病」の病因と同一のものであつたかいなか

は、きわめて興味ある問題である。ガーナのウイルスがないので、ウイルスについて直接比較検討することはできないが、もし流行の前および後にとられたガーナ人の血清があれば、間接的ではあるが、この問題についての手がかりをつかむことができる。著者らはそのような少数の血清を日本にもちかえっていたので、これらについて甲野博士より分与を受けたAHCウイルスに対する中和抗体価を測定した。その結果、ガーナの「アポロ一一病」とわが国の今回の新型流行性出血性結膜炎とは同一の病因（AHC ウイルス）によるものであつたことを強く示唆する成績が得られたので、これらについて報告する。

材料および方法

被検ガーナ人血清は、流行前の一九六八年一二月に採取した六五件、および流行後の一九七〇年から七一年にかけて種々の目的で採取した一四五件であつた。これらの血清は凍結保存されたが、これまで数次にわたり融解がくりかえされた。血清は使用直前五六度Cで三〇分間非動化した。年齢区分は〇～一〇歳、一一～二〇歳、二一～三〇歳、および三一歳以上の四つとした。年齢不明のものの成績は、総平均の算出にのみ使用した。

使用した抗原ウイルスは、国立予研の甲野博士より分与を受けたAHCウイルスの YC-71-670 株（北海道）であつた。これをサル腎細胞で増殖させたのち使用した。中和試験に用いた細胞は、当教室で継代しているヒト胎児肺二倍体細胞（HEL-10 および HEL-11）であつた。増殖培地には一〇%牛血清加イーグルの MEM, 維持培地には一%仔牛血清加の同培地を使用した。

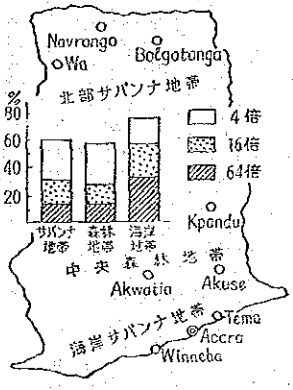
中和試験は、100TCD$_{50}$/0.1ml のウイルス液と等容量の稀釈血清とを混和し、三七度Cで二時間、ついで四度Cで一夜反応させ、その〇・二mlずつを各血清稀釈段階につき二本ずつの HEL-10（または HEL-11）細胞に接種し、三四度Cで静置培養を行ない、ウイルス対照がちょうど 100TCD$_{50}$ 値を示した三、四日目に最終判定を行なつた。血清稀釈は四倍段階稀釈とし、中和抗体価は五〇% CPE 阻止の最高血清稀釈度をもつて示した。

「アポロ11病」流行前後におけるガーナ人の AHC 中和抗体の年齢分布

	年代	被検例	陽性数 4倍	16倍	64倍
流行前（1968年12月）	0～10	2	0	0	0
	11～20	16	7(44)	2(13)	1(6)
	21～30	17	2(12)	0	0
	31～	10	2(20)	0	0
	不明	20	4(20)	2(10)	1(5)
	計	65	15(23)	4(6)	2(3)
流行後（1970～1971年）	0～10	25	18(72)	13(52)	10(40)
	11～20	35	21(60)	11(31)	5(14)
	21～30	31	21(68)	13(42)	6(19)
	31～	34	19(56)	9(27)	2(6)
	不明	20	15(75)	5(25)	1(5)
	計	145	94(65)	51(35)	24(17)

（ ）内はパーセント

ガーナにおける「アポロ11病」流行後の AHC 中和抗体の地理的分布



実験成績

表に示したように、流行前の一九六八年一二月に採取した六五名のガーナ人については、全体として抗体保有のレベルが低く、四倍スクリーニングで二三%、一六倍で六%、六四倍ではわずか三%が陽性であつた。これに対して流行後の一四五件の血清については、四倍スクリーニングで六五%、一六倍で三五%、六四倍では一七%が陽性であり、流行後の抗体レベルの著明な上昇がみとめられた。

年齢別にみた場合、流行後の四倍スクリーニングでの陽性者は、〇～一〇歳ですでに七二%に達し、加齢による陽性率の増加はなくて、むしろ三一歳以上で五六%に減少した。六四倍スクリーニングでは、〇～一〇歳の年齢層がもつとも高く四〇%が陽性であつたが、年齢とともに下降し、三一歳以上では六%に減少した。これらに対して流行前の抗体保有レベルは全体的に低く、一一～二〇歳の年齢層でやや高い抗体保有率がみとめられた。

流行後の血清のうち被検者の居住地の判明した一三〇名について、北部サバンナ地帯（四五名）、中央森林地帯（四八名）、および海岸サバンナ地帯（三七名）にわけて、それぞれ抗体保有率を算出した。その結果、図に示したように、海岸サバンナ地帯が他の二つの地帯よりもやや抗体保有率が高かつた。

考察

今回の報告は限られた材料についての観察ではあるが、ガーナに突如発生した原因不明の「アポロ一一病」と、それから約二年後にパンデミーの一環としてわが国に流行した急性出血性結膜炎（AHC）との関連を求める上で、きわめて貴重な事実をふくんでいる。

一九六九年六月、ガーナで急性の出血性結膜炎が爆発的に発生し、八月に

は極期に達し、一〇月頃から下火となり、その年のクリスマス頃には一応おさまつた。この流行発生の約半年前の一九六八年一二月に著者らの一人が、たまたまガーナを訪問した。その折、首都アクラにあるコレブ教育病院の血清検査室に送られたガーナ人血清を採集していた[5]。そして、一九六九年一〇月、流行のさなかに再びガーナを訪れ、七一年七月まで滞在した。この間に様々の目的で採取したガーナ人血清の一部を日本にもちかえつていた。かくして、数は少ないが偶然にも「アポロ一一病」の流行前と流行後のガーナ人血清を手もとに確保することができた。これらの血清について、日本の流行例から甲野博士らによつて分離されたAHCウイルスに対する中和抗体を測定し、すでに述べたような成績が得られたが、その意味と問題点について考察したい。

まず第一の要点は、ガーナで発生しその後のパンデミーの発端と考えられている「アポロ一一病」と日本で流行したAHCとの関係についてである。「アポロ一一病」流行前と流行後のガーナ人におけるAHCウイルスに対する中和抗体保有レベルは著明に上昇し、この間にガーナにおいてAHCウイルスによる広範な感染（流行）のあつたことは明白である。このことは日本で流行したAHCと原因不明に終つたガーナの「アポロ一一病」とは、ともに同じAHCウイルスによるものであつた可能性がきわめて強いことを示す。ウイルスについて直接比較検討することはすでに不可能となつているが、今回の血清学的データと疫学状況、臨床症状等から、一九六九年六月ガーナで発生した流行性出血性結膜炎（アポロ一一病）と、その後広範な地域に三年間にわたるパンデミーをひきおこした原因ウイルスは、ほぼまちがいなくAHCウイルスによつて代表されるものであろうと考えられる。

第二の問題点は、流行後の抗体の年齢分布と本疾患の好発年齢層とが一致しない点である。日本でも、ガーナでも本疾患の好発年齢層は成人であり、小児の症例は少なかつたといわれている[2,5]。これに対して、流行後の中和抗体の年齢分布は感染（おそらく初感染）が一〇歳以下の小児の間で優位であつたことを示している。このようなことから、AHCの発症は何らかの方法ですでに感作状態にある個体が「眼」という局所の再感染によつておこるのではないかと想像される。そして、これはまた極端に短い潜伏期の問題と相まつて、本疾患におけるアレルギー反応の関与をにおわせるものである。

第三の問題は、第一および第二の問題点の解釈にも大きくひびいてくるが今回著者らの測定した中和抗体が果して完全に従来の意味における中和抗体であるかどうかという問題である。この問題への最初の発端は、甲野博士らの成績[3]と著者らのそれとがあまりにもひらきすぎているのに気づいたことから来ているが、これには、慎重に検討しなければならない実験上の問題がふくまれているように思われる。今回は、ただ方法上の二つの違いについて指摘するにとどめる。

一つは使用した細胞の違いで、甲野博士らはサル腎細胞を、著者らはヒト胎児肺細胞を用いている。第二の相違点は、三七度Cにおける中和時間の長さである。甲野博士らは一時間、著者らは二時間行なつている。なお、この問題については目下詳細を検討中なので、いずれ報告する予定である。

最後に、流行後のガーナ人の間の中和抗体の地理的分布についてであるが、抗体保有率の高かつた海岸サバンナ地帯は、交通、産業、文化等をふくめて、他の二つの地帯にくらべてより近代化の進んでいる地帯であることを付記しておく。

まとめ

一九七一年八月頃から約半年にわたつてわが国に流行した、急性出血性結膜炎（AHC）から分離されたAHCウイルスを抗原とし、一九六九年六月から約半年にわたつてガーナで流行した「アポロ一一病」の流行の前に採取された六五件、および流行後に採取された一四五件のガーナ人血清について中和抗体を測定し、次のような成績を得た。

(1)「アポロ一一病」流行後のガーナ人血清中にAHCウイルスに対する中和抗体が高率に、しかも高いタイターで証明され、日本のAHCとガーナの「アポロ一一病」は同一の病因、すなわちAHCウイルスであつたことを強く示唆する。

(2)流行後における抗体の年齢分布は、初感染が一〇歳以下で成立していることを示すにかかわらず、発症例の大部分が成人であつたこと、さらに潜伏期がきわめて短い点などから、本疾患におけるアレルギー反応の関与が想定される。

本研究の遂行にあたり、抗原ウイルスの分与を賜り、また終始激励を賜つた国立予防衛生研究所中央ウイルス検査部部長甲野礼作博士、およびサル腎細胞の御恵与を賜つた同研究所腸内ウイルス部部長多ケ谷勇博士に対して深甚の謝意を表する。（終）

1) 甲野礼作：日本医事新報，2502：13，1972. 2) 内田幸男，他：日本の眼科，119：3，1972. 3) Kono, R, et al.: Lancet, June, 3: 1191, 1972. 4) Chaterjee, S, et al.: Ghana Med. J, 9: 9, 1970. 5) Chaterjee, S, et al.: Brit. J. Ophthal., 54: 628, 1970. 6) Akinsete, E. O.: J. Nigeria Med. Ass., 7: 46, 1970. 7) Parrott, W. F.: Practitioner, 206: 253, 1971. 8) Minami, K., et al.: Fukushima J. Med. Sci., 19: 7, 1972.