Attended to F. P. de Caires' thesis

Reprinted from The B.G. Medical Annual, 1943.

SULPHAPYRIDINE THERAPY IN PNEUMONIA IN CHILDREN. *

BY

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This investigation was undertaken in the Children's Unit of the Western General Hospital, Edinburgh, during 1939. One hundred cases were studied, and these included a few cases of bronchopneumonia as well as the more common alveolar (lobar) pneumonia. Estimations of the blood-concentration of the drug were not undertaken as at that time no relatively simple method was available. Records in each case include daily leucocyte count, estimation of the blood sedimentation rate and a full clinical examination.

Three distinct methods of administration were investigated :—

(1) Where the drug was administered immediately on admission, continued during the febrile period and then tailed off gradually.

(2) Where the drug was withheld for some hours after admission, to meet the argument that the dramatic fall in temperature due to the chemotherapy was in any way associated with the sharp but transient fall sometimes experienced on admission to hospital.

* Thesis submitted for Dawson Medal.

(3) Where the drug was discontinued as soon as the temperature fell to normal.

MODE OF TREATMENT.

On admission a full case history was taken, and this is given in abridged form. A complete clinical examination was then carried out, including an X-ray photograph, which was taken as soon as possible. The dose of sulphapyridine to be administered was decided upon according to the age and weight of the child, and the gravity or otherwise of its condition; double this "standard" dose was then given, the same amount repeated in four hours, and then the "standard" amount repeated four-hourly, omitting one dose at midnight if the child was asleep. This routine was carried on until the temperature fell to normal. The drug was then "tailed off" by first reducing the frequency of the doses, then the amount. In the second group of cases sulphapyridine was withheld for twenty-four hours after admission. In some of these cases the temperature did not fall, in others it fell for a short while after admission, then rose again.

In substance, the remaining part of the therapy consisted in placing the child's cot or bed at the open window, in pushing the administration of fluids, and in giving sedatives when required. As will be seen this usually resulted in a crisis about twenty-four hours after administration of the drug was started, with concomitant marked improvement in the clinical condition, uneventful convalescence, and complete resolution of the consolidation. The effects of departure from this routine will be seen in the second and third groups of cases.

DOSAGE AND ADMINISTRATION OF THE DRUG.

No absolutely hard and fast limits of dosage were laid down, the clinical condition, as well as the weight and age of the child, being taken into consideration.

Broadly speaking the following table was used as a basis and a guide:—

Age of child.	*Given at once*	*In 4 hours.*	*4-hourly after*
Up to 3/12	½ Gm.	¼ Gm.	⅛ Gm.
3/12 to 1 yr.	¼ Gm.	½ Gm.	¼ Gm.
1–3 yrs.	½–1 Gm.	½–1 Gm.	¼–½ Gm.
3–6 yrs.	1 Gm.	1 Gm.	½ Gm.
6–12 yrs.	1–2 Gms.	1–2 Gms.	½–1 Gms.

The white blood count (referred to as W.B.C.) and the clinical response, as well as the presence or absence of toxic manifestations, were the guides to the length of administration, the quantities given, and whether or not the usual mode of therapy was followed.

The usual tablets of 0.5 Gram were used. In very small infants the tablets were finely crushed and given in a teaspoonful of milk or glucose-water. The drug was never given in the bottle-feed. For bigger children unable to swallow the tablets, they were coarsely crushed and given in a tablespoonful of milk. Older children took the tablets quite readily.

It cannot be stressed too forcibly that children, as a general rule, displayed none of the nausea and consequent reluctance to take the drug so often experienced in adults. In a few of the older children slight reluctance was readily overcome by a little coaxing, with or without some change in the mode of administration, *e.g.*, suspending the crushed tablet in milk instead of glucose-water, or tea, or cocoa.

As regards the total amount of sulphapyridine given, one case aged 4 years took 36 Gms. in three weeks, another aged 11 years had 42 Gms. also over a period of three weeks, another aged 1¾ years had 8 Gms. in three days, all without any sign of toxic effects.

One child, JOHN K., a little over one year of age, who was admitted with pneumococcal pneumonia, a bilateral Haemolytic Streptococcal otitis media, and obviously in a very toxic state, was given the drug in 1–1–½ Gm. dose scale to produce a quickened effect, without the least upset.

DIETARY RESTRICTIONS.

Following the general rules in the treatment of a febrile illness like pneumonia, fluids were given in the early stages in as large amounts as possible. Apart from the routine nature of this measure, however, it was found that in cases where fluids were well taken, the response appeared to be better. Whether this is just a general effect, or is connected in some specific way with the drug, cannot be stated as a result of this report. It may be noted, however, that other investigations revealed that dilution of the blood would not lower the effective concentration of sulphapyridine below the active level required to be effective against the organisms. If a purgative was required magnesium sulphate was avoided.

When more solid fare could be taken, one egg daily was given without any ill effects whatever. It is noteworthy that the shortened period of the illness brought about by this form of chemotherapy makes it very much easier to avoid any article of diet that may be suspect. Onions, for example, hardly enter the list of foods approved for a convalescent case of pneumonia; even eggs can be avoided during the few days on which the drug is being given.

RESPONSE OF THE TEMPERATURE TO SULPHAPYRIDINE.

The average day of illness on which the cases were admitted to hospital was just after the third day, and it is found that in the average case, uncomplicated by development of an empyema or other disease, the crisis occurred 4.43 days after the onset of the illnes. The average interval after the first dose of sulphapyridine until the crisis was 1.04 days.

Definite clinical improvement occurred about 8–10 hours after the first dose. This tallies with estimations of the blood-level of the drug, which reveal an effective concentration with adequate dosage after about six hours. In a few instances, where children contracted the disease while in the wards, a remarkable response with an almost unbelievably rapid recovery took place.

In the first two groups of cases, where the drug was "tailed off" during about two days after the crisis, the temperature remained down. In Group III, where the sulphapyridine was abruptly stopped as soon as the temperature fell, there followed another rise, which invariably proved to be less responsive to further administration than at first, and usually the temperature fell for the second time by lysis, and not by crisis. The temperature graph was found to be an accurate gauge to the clinical response, the patient's condition improving as the temperature fell, and as dramatically.

LEUCOCYTIC RESPONSE.

The invariable response of the white blood cells, in cases with an average leucocyte count, was a sharp fall, the graph of which corresponded closely with the temperature crisis. This is one of the features of this investigation. In the short series of similar graphs from cases not under chemotherapy, it was found that there is a like similarity, but not so exact in nature.

The fall in the white cell count was very dramatic indeed, and in most cases the figure dropped below the average for the age period. The curve soon climbed back to normal levels, however, and in the case of a serious drop below normality, cessation of the drug resulted in an immediate response. This "biphasic" response was more the rule than the exception, and also forms one of the salient points in this report.

In direct response to the drug, on the other hand, the case with a poor leucocyte response, or none at all to the disease, there followed a RISE in the white cell count. This in turn was followed by the usual fall. The type of case with an initial low count was invariably the grossly toxic patient, with signs of central and peripheral circulatory failure; while the mechanism of this paradoxical rise in the leucocytes is not obvious, it may be suggested that the drug releases the toxic brake on the bone-marrow response to the disease.

It may be stated here that I had the good fortune to corroborate this finding in several adult cases after this investigation was concluded. Standard equipment and technique were used in the estimation of the leucocyte count, and any figure that appeared out of the ordinary was checked and re-checked; in this way the figures given are probably within the usual allowance for experimental error.

Full differential counts were not undertaken, as a large enough series from which any useful conclusions could be drawn was not available. The few actually done, with a special eye to the monocyte count, did not reveal any noteworthy feature.

RESPONSE OF THE BLOOD SEDIMENTATION RATE.

The Blood Sedimentation Rate (B.S.R.) was estimated by means of a micro-pipette method, similar to the ordinary blood-counting pipettes for white and red cell

counts. The instrument consisted of a metal suction apparatus, into the foot of which the pipette was fitted; blood was obtained from the lobe of the ear as for a blood-count, and a column drawn up to a graduated scale. Dilution with a solution of 2½% Sodium Citrate was then carried out by sucking up the citrate to another mark, mixing in a bulb on the pipette. The column was then allowed to stand for one hour, and the reading made in millimetres.

The maximum reading on this scale was 45 mms., and a series of controls shewed that the readings on this micro-scale corresponded to the more used 200 mm. tube in the ratio of 1: 3.5. The normal reading in health was in the region of 2–3 mms.

It will be seen that the graph of the B.S.R. fell slowly with the diminution of the toxaemia; the fall was a more gradual and a more delayed one compared to the fall in white cells.

A consistent and interesting finding is that, in spite of the earlier crisis and clinical improvement in cases treated with sulphapyridine, the blood sedimentation rate fell more slowly than in the untreated cases. For instance, most of the case charts given here read up to 12–18 days of the disease; the average level to which the B.S.R. fell in treated cases is 20 mms., while in the untreated, the level is about 10 mms.

The above finding is of especial interest in view of the two cases in which sulphapyridine was administered to apparently healthy children; the B.S.R. rose to a slight extent, and the W.B.C. fell from the basic figure established beforehand. It is realized that two cases are of little value, and the shortage of material and urgent need of as quick a bed "turnover" as possible, made it impossible to keep children in hospital for long enough periods after the acute stage of the illness was over, to carry out further investigations. The observed facts are merely stated here as being of interest.

As in the estimation of the blood-counts, a standardized technique, with standard reagents, was employed, to cut down experimental error to a minimum. With the micro-method used it is clear that smaller variations must have more significance than on the 200 mm. tube scale.

RESOLUTION OF THE CONSOLIDATION.

In every case in this series resolution was complete. The average interval between the onset of the disease and the date of complete resolution was 13.75 days. The figures for the previous three years, in the same wards of the hospital, give an average period of 18.22 days. I may state that the adult medical wards gave the following intervals :—Treated cases for the same year during which the investigation was being carried out—17.37 days: untreated cases over the previous three years —17.08 days. The average interval between the date on which the sulphapyridine was begun, and the date of complete resolution was 10.4 days, in the present series.

It is worthy of note that the series under consideration included the following :—

(a) A chronic bronchitic where resolution took place on the 20th day; interval on the drug 19 days.

(b) Cases with two separate patches of consolidation where resolution occurred on the 16th and 17th days, and the intervals after starting the drug were 13 and 16 days respectively.

TOXIC EFFECTS OF SULPHAPYRIDINE THERAPY.

It must be stated at the outset that the salient feature in this respect is the LACK of toxic effects. Even in children who took the drug over relatively long periods, and in large doses, ill effects were marked by their absence.

The following instances did occur, however, and are definitely attributable to the administration of the drug :—

(1) A HAEMORRHAGIC RASH in a very young baby, a mongol of three weeks, who had a severe bronchopneumonia. The drug was stopped, and the infant made an uninterrupted and complete recovery.

(2) VOMITING occurred in a child of 5 years who was given full adult dosage for 24 hours in what appeared to be a succesful effort to bring her very quickly under the influence of the drug, as she was gravely ill on admission. She had been vomiting before admission, but probably from an acidosis which was counteracted after coming into hospital.

(3) MARKED CYANOSIS was a feature in one girl, whose illness was far from typical. She came in on the 14th day of the illness, the family doctor suspecting a respiratory illness all the time, but failing to discover any clinical signs on careful and repeated examination of the chest.

(4) TRANSIENT BUT RECURRENT CYANOSIS in a boy who developed an empyema.

(5) APPARENT SENSITIVITY TO U-V LIGHT in a little boy who displayed a very marked reaction to a minute exposure two weeks after cessation of sulphapyridine therapy. This same child was exposed to sunlight while on the drug, with no ill effects. No other case was allowed U-V light therapy while on sulphapyridine.

(6) SHARP FALL IN WHITE CELL COUNT, if this can be considered under this heading. An initial fall below the average age-group figure has already been noted, but in some cases this

fall was well beyond the average for the series. No matter how sharp the drop, however, correction was immediate on stoppage of the drug.

While this investigation was being made much interest was centred on the alleged increased incidence of empyema since the use of the drug in pneumonia. The figures for the year under consideration were well under the average for the past three years.

Contraindications.

As has already been noted the lack of toxic effects is one of the features of this investigation.

The following were, however, taken as absolute contraindications to the continuance of the drug:—

(1) Persistent vomiting, in spite of a change in the mode of administration, and the failure of such recommended drugs as Sodium Bicarbonate to control the vomiting.

(2) A very sharp fall in the white cell count from a high figure to well below average limits.

(3) A severe rash.

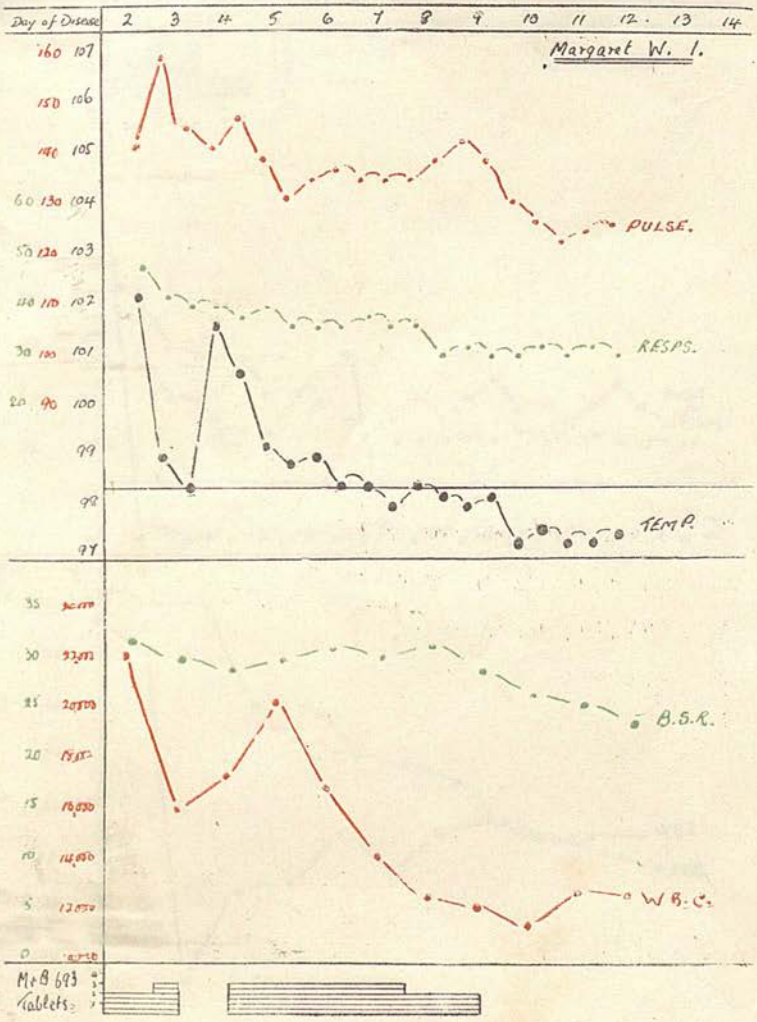
(4) Deep persistent cyanosis.

Case Histories.

Chart—(David G.)

Typical history with sudden onset. Physical signs revealed right basal consolidation. Sputum "rusty," containing pneumococci, Type 2.

Previous Illness:—1929 pneumonia; 1933 pneumonia; 1934 pneumonia; 1936 measles.


Day of Disease
2 3 4 5 6 7 8 9 10 11 12 13 14
Margaret W. I.
160 107
150 106
140 105
60 130 104
50 120 103
110 102
30 100 101
20 90 100
99
98
97
PULSE.
RESPS.
TEMP.
35
30
25
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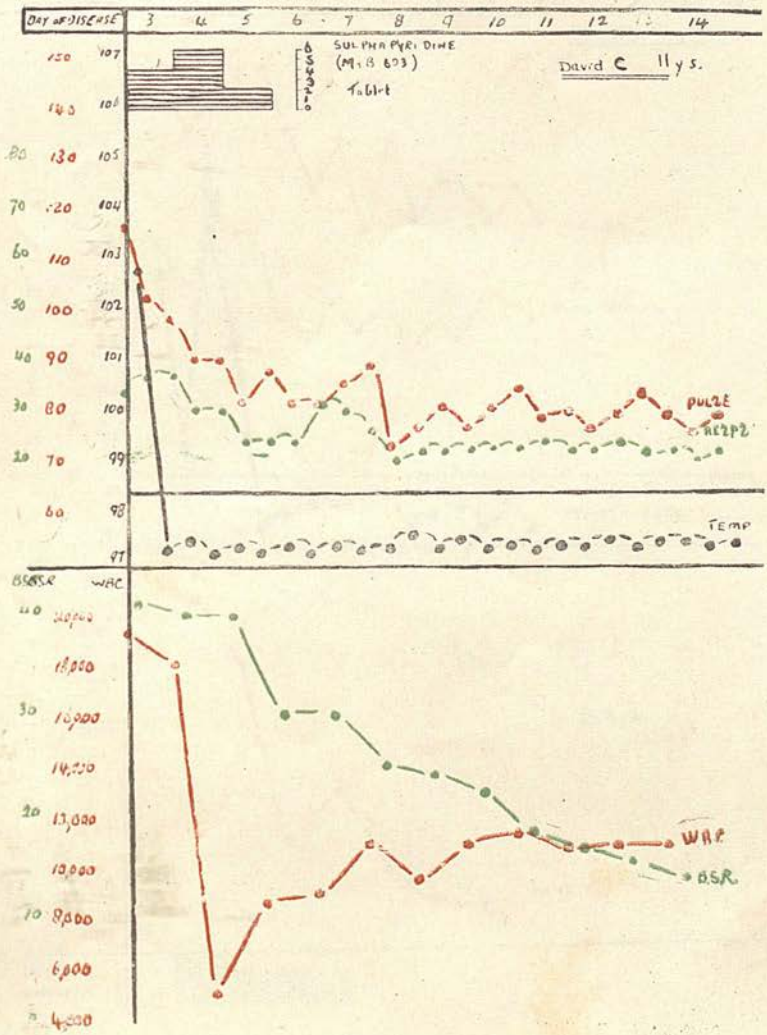
10
5
0
B.S.R.
W.B.C.
M+B 693
Tablets


DAY OF DISEASE
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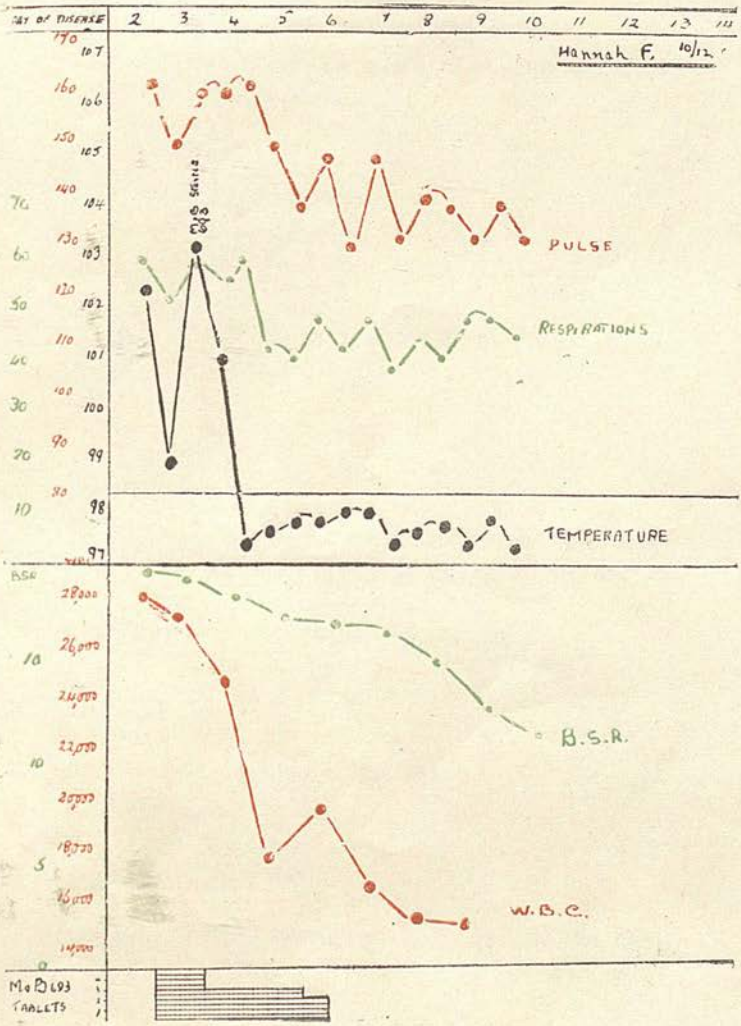
DAY OF DISEASE
2 3 4 5 6 7 8 9 10 11 12 13 14
Hannah F. 10/12
PULSE
RESPIRATIONS
TEMPERATURE
B.S.R.
W.B.C.
M & B 693
TABLETS

Past History : Premature, 3 weeks.

Mother had toxaemia during pregnancy.

Never breast-fed; given cow's milk plus cod liver oil and orange juice.

Frequent colds.

Milestones :—Teeth at one year.

Spoke at ten months.

Walked at one year.

Crisis on third day, with an uneventful recovery. Resolution complete on eighth day.

Toxic effects of the drug :—nil.

Chart—(Hannah F.)

Drug withheld for 24 hours after admission; the temperature fell on admission but rose again to 103.4°.

Causative Organism :—Pneumococcus, Group 4.

Toxic effects of the drug :—nil.

Crisis on fourth day.

Resolution complete on the tenth day.

Uneventful recovery.

Chart—(Margaret W.)

Case in which the drug was stopped as soon as the temperature fell. A further rise immediately followed, with the final drop more by lysis.

Organism :—Pneumococcus, Group 4.

Toxic effects :—nil.

Resolution complete on the tenth day.

SUMMARY.

One hundred cases of pneumonia treated with sulphapyridine were investigated. There was one death (a case of bronchopneumonia seen on the 34th day of illness, organism Streptococcus viridaus, with terminal vasomotor failure) giving a mortality rate of 1%; the figure for the three previous years, without chemotherapy, was 13.6%.

The reactions of the white cell count and blood sedimentation rate are shown. Clinical improvement occurred about eight hours after administration of the drug, which was well tolerated by the children. Resolution was complete, and recoveries were uniformly uneventful.

A few cases displayed some sensitivity to the drug and the indications are that some patients are hypersensitive to sulphapyridine; as this sensitivity is obvious from the outset ordinary care will prevent any catastrophe. The danger if the drug is not used under medical supervision is no less obvious.

The comparatively short interval of administration made dietary restrictions easy and readily adaptable.

There was a marked absence of toxic effects as a result of the administration of sulphapyridine.

ACKNOWLEDGMENTS.

My thanks are due to Professor Charles McNeil for permission to undertake this investigation, and for his sustained interest in the work. I am also grateful to Dr. Eric Dott and Dr. T. Y. Finlay for their help and guidance. The results achieved were in great measure due to the high quality of the nursing-care given by Sister M. E. Russell and her staff.

Reprinted from the B.G. Medical Annual, 1943.

IRON THERAPY IN SEVERE CUTANEOUS LEPROSY.

BY

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This hospital is situated on the Atlantic seaboard of the Colony. There are about 400 in-patients and 600 out-patients, the latter being treated at various clinics. During the routine examination of patients, haemoglobin readings were found to be so abnormally low that this investigation was undertaken over the period January 1941 to February 1942.

The following groups were examined :—

GROUP "A"—A "Control Group" consisting of 21 hospital attendants and non-leper out-patients seen for trivial ailments. Average : 85.24%.

GROUP "B"—28 Severe Cutaneous Cases, all L2* and L3* types, who failed to improve on routine anti-leprosy treatment, in whom no intercurrent disease could be found. Average : 50.75%.

GROUP "C"—56 able-bodied patients of all types, many of whom were doing hard physical work, and all of whom were doing at least ordinary physical work. Average : 62.85%.

GROUP "D"—23 discharged patients living at Lancaster Village, just outside the hospital, who obtain raw rations from the institution. In all cases this diet is appreciably supplemented. These patients were practically all "burnt out" neural cases. Average : 68.69%.

GROUP "E"—16 discharged patients living in Georgetown, being mixed types in whom the disease had been arrested. A comparison of their haemoglobin readings and financial status revealed an obvious relationship between the two. Average : 72.20%.

GROUP "F"—16 children in the Lady Denham Home, outside the hospital, in whom the disease had been arrested. Average : 64.50%.

GROUP "G"—43 children with active leprosy, whose condition varied from "improving satisfactorily" to "very bad." Average : 62.55%.

The haemoglobin estimations were carried out on the Haemoglobin Scale, Tallqvist's pattern, checked against the Sahli Haemoglobinometer. This method was used for obvious reasons of time and convenience. Blood films were examined and white and red cell counts made, as well as reticulocyte estimations. The universal finding was a simple microcytic hypochromic anaemia, of varying severity, and a Colour Index range of 0.6 to 0.7 as a general rule. No case was included in this investigation in which intercurrent disease or sepsis existed. Strict adherence to this principle maintained as near an uncomplicated field as possible. Although septic sores in L2* and L3* cases healed without special local treatment in cases under iron therapy, and their general condition improved, even these were not included. Particular

attention was given to hookworm infestation, and unless otherwise stated this was either absent or had been eliminated some considerable time before.

Treatment was begun with a mixture containing Ferri et Ammon. Citrate gr. 30 t.d.s. A striking feature was the rapid response to this form of therapy up to a level of 65—70%, after which further response could be obtained with Ferrous Sulphate gr. 5 t.d.s. This finding is, of course, not new. With the rise in haemoglobin clinical improvement followed, after a lag interval. This is another striking feature of the investigation, and was particularly noticeable in the very severe cases who were going steadily down hill in spite of vigorous anti-leprosy treatment, and the absence of any discoverable intercurrent infection. In fact, the barometer-like nature of the haemoglobin curve attracts the attention at once. It is worthy of note that the hospital stock mixture containing Ferri et Ammon. Citrate gr. 30 is given to patients from time to time, t.d.s, as a general "tonic."

Patients were able to maintain the higher levels of haemoglobin percentage after the iron therapy, *i.e.*, the intensive dosage, was discontinued. The influence of diet is difficult to assess, but Groups "A," 'D," "E" and "F" are comparable, and those on hospital diet are lower. Very low readings were found in East Indians whose diet contains ample quantities of "greens" but these are cooked, and the other articles of diet are sadly lacking in essential factors. In Group "G" added "greens" in suitable form and quantities produced a rise in the average reading from 62.55% to 69.63%, in a group to whom no iron was administered, in three months.

The Blood Sedimentation Index was obtained in the following way. Citrated venous blood was drawn up into a 200 mm. tube and readings were taken after 1½ and 2½ hours; the mean of these two gave the Index. Routine readings were only done after August 1941.

(See Chart opposite).

This chart illustrates a typical response to iron therapy by severe L2* and L3* cases, and while no specific action can be claimed for just a rise in the haemoglobin, the often spectacular changes appear to be out of proportion to be a mere improvement in the oxygen-carrying power of the blood. The results illustrated were repeatedly obtained, though with a less dramatic rapidity in the less severe cases. In two cases positive nasal smears became negative in the absence of specific local treatment after a considerable rise in the haemoglobin. One of these relapsed, however, following a severe bout of Benign Tertian Malaria which induced a "reaction."

Throughout the investigation routine specific anti-leprosy treatment was carried out with no interruption, but it is only fair to state that in all of the bad cases under observation this had failed to produce any improvement over a prolonged period. This routine consisted of the intramuscular and intradermal injection of the esters of hydnocarpus oil, the application of suitable strengths of trichloracetic acid to the skin, and the oral administration of an alkaline mixture plus fluorescin by the intravenous route during "reaction."

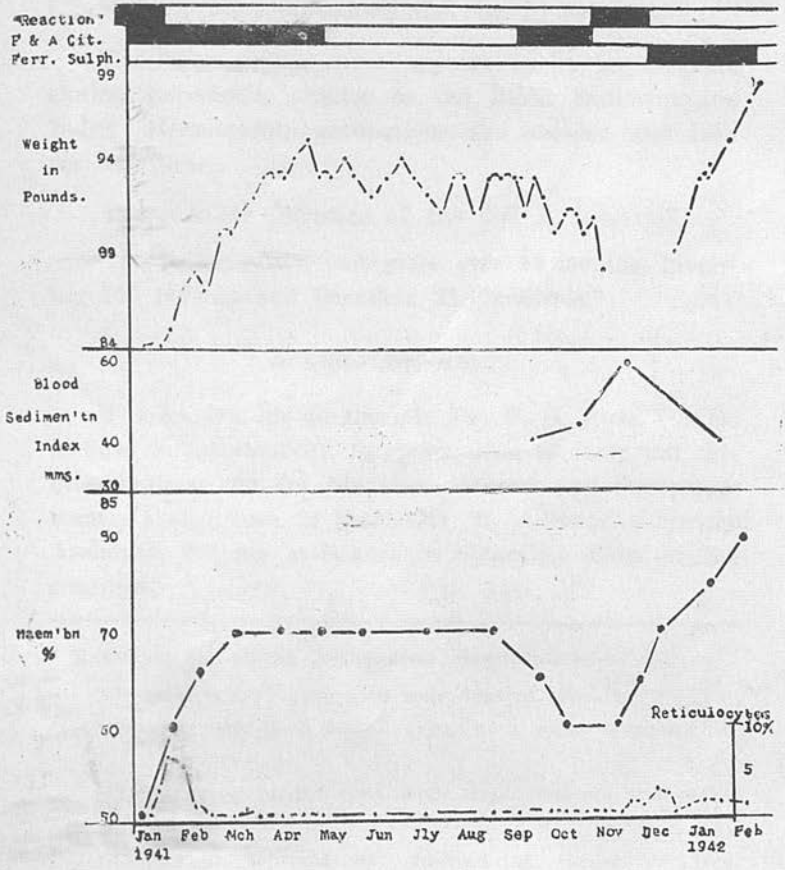
The possibility of a relationship between these results and the temporary improvement effected by repeated blood transfusions should not be overlooked. Although the numbers involved in this investigation are small, the results appear to furnish at least a basis for further clinical tests.

SUMMARY.

Abnormally low haemoglobin readings have been found in patients suffering from leprosy, particularly the lepromatous form, in the absence of intercurrent disease and secondary septic complications. This is particularly marked in the L2* and L3* cases where patients fail to improve on routine treatment over a prolonged period.

CHART J.C. Boy aged 15 years, Chinese.

Severe lepromatous type, subcutaneous nodules.



Response to Ferri et Ammon. Citrate is immediate and rapid up to a level of about 65—70%, and further gain follows the administration of Ferrous Sulphate. Clinical improvement follows the rise in haemoglobin.

The haemoglobin curve appears to be an accurate clinical barometer, similar to the Blood Sedimentation Index. Haemoglobin estimations are simpler and take far less time.

The possible influence of the diet is indicated.

The investigation took place over 14 months, involving 195 persons and including 21 "controls."

ACKNOWLEDGEMENTS.

Thanks are due to the late Dr. F. G. Rose, O.B.E., Medical Superintendent, for permission to carry out this investigation and for his keen interest and encouragement. I also wish to thank Mr. R. Adhin, Laboratory Assistant, for his assistance in obtaining many of the readings.

* Based on the Cairo Conference's classification:—

N1—mild neural type, with some loss of sensation.

N2—more advanced neural type, with some muscular involvement.

N3—advanced neural type, with trophic ulcers and severe deformities.

L1—(using "lepromatous" instead of the older term "cutaneous") mild localised skin lesion or lesions.

L2—more widespread skin lesions.

L3—diffuse or infiltrative type of skin lesions. The worst type is the case with widespread subcutaneous nodules.

Combinations of the above types occur, as well as the Minor and Major Tuberculoid groups, formerly classed under "neural".

Reprinted from
THE PUERTO RICO JOURNAL OF PUBLIC HEALTH AND TROPICAL MEDICINE
Volume 22, No. 4, pages 405 to 415
June 1947

DDT and *Aedes aegypti* Control in British Guiana[1]

By P. F. DE CAIRES

From the British Guiana Yellow Fever Service[2]

In February 1945, Symes and Hadaway[3] initiated experiments to determine the effect of DDT residue-sprays on *A. darlingi*, the malaria vector in British Guiana. Later in the year, it was decided to carry out a further experiment to determine the efficacy of the drug in *Aedes aegypti* control and to obtain data that would permit a comparison between the costs of DDT spraying and routine anti-*Aedes* control measures.

British Guiana lies in a strategic position as regards yellow fever, since it is the last place of call for northbound air and sea traffic from the South American continent. In order to protect the thickly populated non-immune coastlands, *Aedes aegypti* control was started in 1939 as a joint Rockefeller Foundation-Colonial Government project. The absence of a piped potable water supply and the presence of a very complex system of rain water storage for drinking purposes create problems that have made *Aedes* control abnormally difficult. The war-time shortage of sealing and other materials aggravated the position still further.

Dr. George Bevier, staff member of the International Health Division of the Rockefeller Foundation, then Chief Officer of the Yellow Fever Service and Director of the Malaria Research Unit, and Dr. George Giglioli, Malariologist, arranged for the release, in August 1945, of the required amount of DDT and the use of the only power sprayer then available.

Site of the experiment. The adjoining villages of Sparendaam and Plaisance, situated along the coastal road about six miles east of Georgetown, were chosen for the experiment. The population of 3,293 live in 1,020 houses, which vary from the typical two-storey city dwelling to the smaller rural cottage (Figs. 1 and 2). Table 1 sets out the conditions obtaining in the sprayed houses, which are all built of wood and elevated off the ground on pillars.

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1. Received for publication September 20, 1946.

2. The British Guiana Yellow Fever Service is financed by the Government of British Guiana, a grant under the Colonial Development and Welfare Scheme, and the Rockefeller Foundation.

3. C. B. Symes and A. B. Hadaway, Initial experiments in the use of DDT against mosquitoes in British Guiana. *In press.*

TABLE 1

Conditions in DDT-sprayed Houses

Area	*No. of Houses*	*Roof*				*Walls*					*Furniture*	
Percent DDT		*Galvanized*	*Board*	*Shingles*	*Combination*[a]	*Papered*	*Painted*	*Plain board*	*White-washed*	*Combination*[b]	*Ordinary*[c]	*Good*[d]
2½	79	19	14	22	24	20	20	16	2	21	66	13
5	228	44	51	66	67	72	72	45	0	39	198	30

[a]A combination of more than one type.
[b]*Ibid.*
[c]Plain wood.
[d]Painted, varnished, or polished.

Drinking water is stored in large and small containers. Rain water is collected from the roofs by means of galvanized gutters, which lead it off into vats, tanks, barrels, or empty gasoline drums. Water from artesian wells is collected and stored in every conceivable type of receptacle, including the ubiquitous clay "goblet," frequently hidden from the prying eyes of inspectors.

As shown in the accompanying map (Fig. 3), the experimental area was divided into four parts: (1) 79 houses were sprayed with a solution (5 percent) made up of one half pound of DDT per gallon of kerosene; 228 houses were sprayed with a solution (2½ percent) made up of one fourth pound of DDT per gallon of kerosene;[4] (3) 713 houses were subdivided into three zones and placed under routine anti-*Aedes* control measures on a 7-day cycle, later lengthened to 14 days as the indices fell; (4) Goedverwagting, a control area, where no anti-mosquito measures were undertaken.

This site was chosen because it was virgin area within easy reach of headquarters in Georgetown. The initial survey showed an *Aedes aegypti* index (percentage of houses with *Aedes aegypti* breeding) of 26 percent. Table 2 gives a list of the water containers in each area.

Equipment and personnel. Powdered DDT (Geigy, containing 72 percent of the para-para-isomer) was dissolved in commercial

4. Mixing of DDT in kerosene was carried out by stirring with a wooden paddle and leaving overnight.

TABLE 2

Water Containers

Container	*Goedverwagting (Control Area)*	*Area*	*DDT Sprayed Percent*	
		Routine Control Measures (Control by Inspectors)	2½	5
Water boxes	0	10	0	0
Vats and tanks	9	96	16	5
Clay containers	33	306	75	29
Barrels and drums	79	216	93	42
Special—In artificial-containers—[a] Out	102 88	2,001 1,408	550 481	192 197
Other containers[b]	45	568	86	69
Tubs[c]	31	343	82	36
Total	387	4,948	1,383	570

[a]Buckets, vases, tin cans in- and outside of houses.
[b]Latrines, drains.
[c]Made of wood and used for washing clothes; often kept wet to prevent shrinking.

kerosene (non-deodorized) to the required strength. The only available sprayer was a Briggs-Stratton gasolene motor-gear pump sprayer, provided with a relief valve and by-pass overflow. The solution was pumped through oil resistant rubber hose, at the end of which a trigger-controlled metal rod was fitted with a nozzle giving a linear jet (Knipe modification) and delivering one sixth British imperial gallons per minute at a pressure of 50 pounds.

Unfortunately, this power sprayer, which had already given long service, proved very unreliable. Frequent breakdowns led to tedious delays, greatly increasing labor costs, as the entire spraying squad was immobilized whenever work had to be suspended. The squad consisted of a supervisor, a sprayer wearing a gauze mask, a mechanic in charge of the pump, and an inspector serving notice of the squad's impending arrival and supervising preparations for spraying.

Technique. Householders were given twenty-four hours' notice and requested to put out all fires and to close all windows and doors before spraying. On the squad's arrival, a check was made to see that these instructions had been carried out. Foodstuffs were then removed to avoid any contact with the spray. Clothing behind doors was also removed so that the entire wall surface could be sprayed. The solution was applied so as to wet, without "running" all walls, jalousies, closed windows and window curtains, low ceilings,

mosquito nets, and the under surfaces of furniture. The interior of cupboards and mattresses were sprayed on request. After spraying, the house was then kept closed for about half an hour. The time most convenient to spray was found to be between 7 A.M. and 1 P.M. Estimated dosage used was 100 mg. of DDT per square foot.

Table 3 gives the data collected for each house.

TABLE 3

Data Collected for Each House Sprayed

House No.	*Dimensions*	*Rough Plan*	*Gallons of Solution Used*	*No. of Residents*	*Type of*
Street and lot	Overall length, width and height of ceiling	Sketch of the rooms	DDT in kerosene	Adults Children	Roof Walls Furniture

Public Cooperation. The public cooperated very willingly. In not a single instance was opposition encountered, no doubt due to the extensive publicity given to the drug. No damage to property resulted from the spraying.

Costs. 79 houses—5 percent DDT in kerosene

A.	87 gallons of kerosene.....B.W.I.		$28.44[5]
	43 pounds DDT at 78 cents per pound.....		33.54
	Transportation, labor, etc.....		36.00
			$97.98
		$1.24 per house	
	Experimental checking (10 months).....		63.00
			$160.98
B.	*228 houses—2½ percent DDT in kerosene*		
	272 gallons of kerosene.....B.W.I.		$88.92
	68 pounds DDT at 78 cents per pound.....		53.04
	Transportation, labor, etc.....		112.00
			$253.96
		$1.11 per house	
	Experimental checking (10 months).....		189.00
			$442.96

5. B.W.I. $4.80 is equivalent to 1 English pound; ~~B.W.I.~~ $1.00, to $1.18 ~~U.S.~~ currency.

C. *713 houses—Routine Aedes Control* (Control of breeding places by inspectors)
10 months.....B.W.I. $1,210.00
$1.69 per house

The price of DDT has varied between B.W.I. $0.78 and $1.59 per pound and is not yet stable. An idea of comparative labor costs may be obtained from the salary scale of inspectors, which varies from B.W.I. $18.00 to $24.00 for new men, plus uniforms and bicyle allowances, to $36.00, or more, for chief inspectors.

Aedes aegypti indices. Figure 4 shows the graph of the indices for the 5 percent and 2½ percent DDT-sprayed areas and the zones under routine control measures. Zones 1 and 3 were placed on a 14-day cycle after the week ending January 19, 1946; zone 2 remained on a weekly cycle throughout the period under review, viz., January 9, 1945 to April 5, 1946.

The 5 percent DDT-sprayed area attained an index of one percent after eight weeks and a zero index, after thirteen weeks. The latter was maintained thereafter. The graphs for the 2½ percent DDT and routine control areas run a parallel course. In the control area, where no anti-mosquito measures were carried out, the index showed no significant variation. In the DDT sprayed areas, foci found were recorded and left undisturbed; no effort was made to eliminate them.

Aedes control with DDT in other areas. In February 1946, Lodge Village, situated in the southeastern corner of Georgetown and bordered on two sides by *A. darlingi* breeding grounds, was sprayed with a 5 percent solution of DDT in kerosene by the Malaria Service. This block of 662 houses is included in the area under routine *Aedes aegypti* control. Since the date of spraying, the village has had, and maintained, a zero *Aedes* index.

In three localized areas in Georgetown itself, where chronic *Aedes* breeding persisted, spraying with 5 percent DDT in kerosene eliminated the breeding after a two-weeks' interval. In all three instances, the spraying was confined to a single house.

Effect of DDT on Culex breeding. Figure 5 illustrates the house indices for *Culex* breeding in the areas sprayed with DDT and under routine control measures.

The predominant *Culex quinquefasciatus* breeds in and around houses but mainly in pit-latrines and drains. As was to be expected, it was far less susceptible to DDT house-spraying than *Aedes aegypti.* Giglioli[6] has demonstrated that adult *Culex* are the most resistant

6. G. Giglioli, Annual Report of the Malaria Service, British Guiana, 1945.

to DDT of the species studied by the Malaria Service. Our figures, based entirely on larval findings, provide supporting evidence.

Improved spraying techniques. Since this experiment was carried out, there has been considerable improvement in the types of spraying equipment, with consequent reduction in the cost of spraying. Specially constructed power sprayers and Cooper pumps, the latter originally designed for spraying cattle, are now in use. Giglioli gives B.W.I. $1.16 as the cost of spraying a rural cottage late in 1945.

DISCUSSION

This experiment clearly demonstrates the great susceptibility of the very domestic *Aedes aegypti* to house-spraying with a 5 percent solution of DDT in kerosene. In the type of houses found in the experimental area, this method of *Aedes* control proved more effective and cheaper than routine anti-*Aedes* control measures, even when carried out with inferior spraying equipment. It is clear that the 2½ percent DDT solution was not effective. In an emergency, spraying with a 5 percent solution of DDT, perhaps coupled with routine control measures, should be the control method of choice.

In the experimental area thus treated, a single spraying has remained effective for ten months. We have yet to determine how long the effect will last. Giglioli has demonstrated that for *A. darlingi* a single spraying gives a 98 percent reduction after ten months; he has since shown that some effect is still evident after sixteen months.

The results obtained would seem to indicate that *Aedes aegypti* eradication, on a large scale, would be more economically attained by spraying with a 5 percent solution of DDT in kerosene than by routine anti-*Aedes* control measures. It is now proposed to spray a large area on each side of the Demarara River estuary as a combined Sugar Estate, Malaria and Yellow Fever Services project. This experiment should provide much valuable information, particularly with regard to costs, and may give an answer to the question of whether or not a single spraying will eradicate *Aedes aegypti.* Any method of *Aedes aegypti* control must necessarily include measures for the prevention of the importation of the mosquito, *e.g.*, by ship, airplane, train, and motor vehicle.

In assessing the efficacy of DDT in this experiment, it must be remembered that the sprayed area is surrounded on three sides by *Aedes aegypti* infested houses. The proposed experiment on the Demerara River banks will provide information on the effects of spraying large and relatively isolated areas.

SUMMARY

The experimental control of *Aedes aegypti* by residual house-spraying with DDT in kerosene is described. A 5 percent solution produced eradication of the species thirteen weeks after spraying, and this effect has been maintained for ten months at a cost of B.W.I. $1.24 per house.

A 2½ solution and routine *Aedes* control measures proved less effective and more expensive, the results for these two methods being roughly parallel.

The effect of the drug on *Culex quinquefasciatus* is also described. As expected, DDT proved less efficient than routine mosquito control measures.

The technique and equipment employed are described; there has been considerable improvement in both since the experiment began in August 1945, with consequent reduction in the cost of spraying.

ACKNOWLEDGMENT

The author is indebted to Dr. H. B. Hetherington, O.B.E., Director of Medical Services, for permission to publish the results of this experiment; to Dr. George Bevier, staff member of the International Health Division of the Rockefeller Foundation, then Chief Officer, Yellow Fever Service, under whose guidance the experiment was begun, and to Dr. George Giglioli, Malariologist, for his help and advice.

FIGURE 1. Types of Georgetown, B.G. Houses in Which Experiment on DDT Control Was Carried Out.

FIGURE 2. Types of Georgetown, B.G. Houses in Which Experiment on DDT Control Was Carried Out.

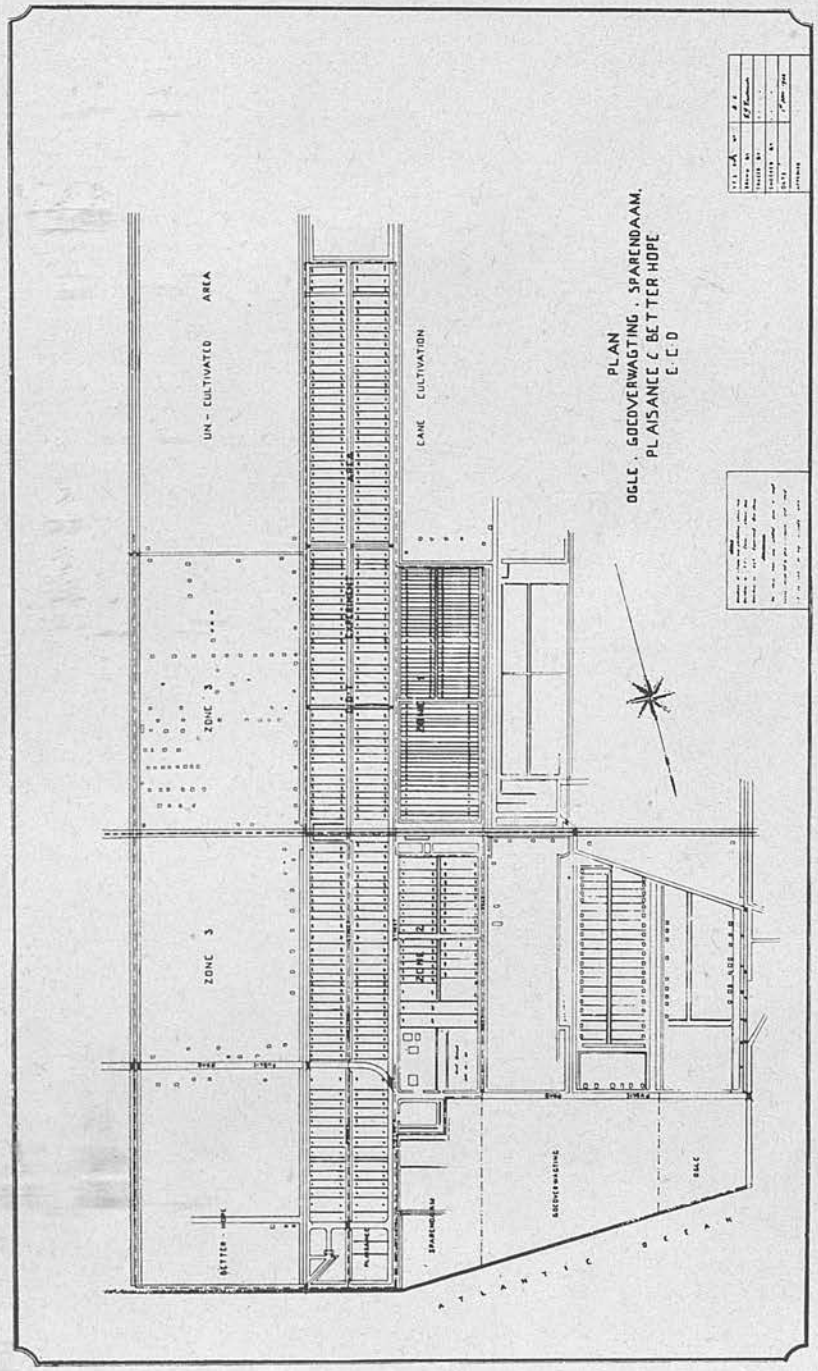

FIGURE 3. Area of Georgetown, British Guiana, Where DDT-Control Experiment Was Carried Out.

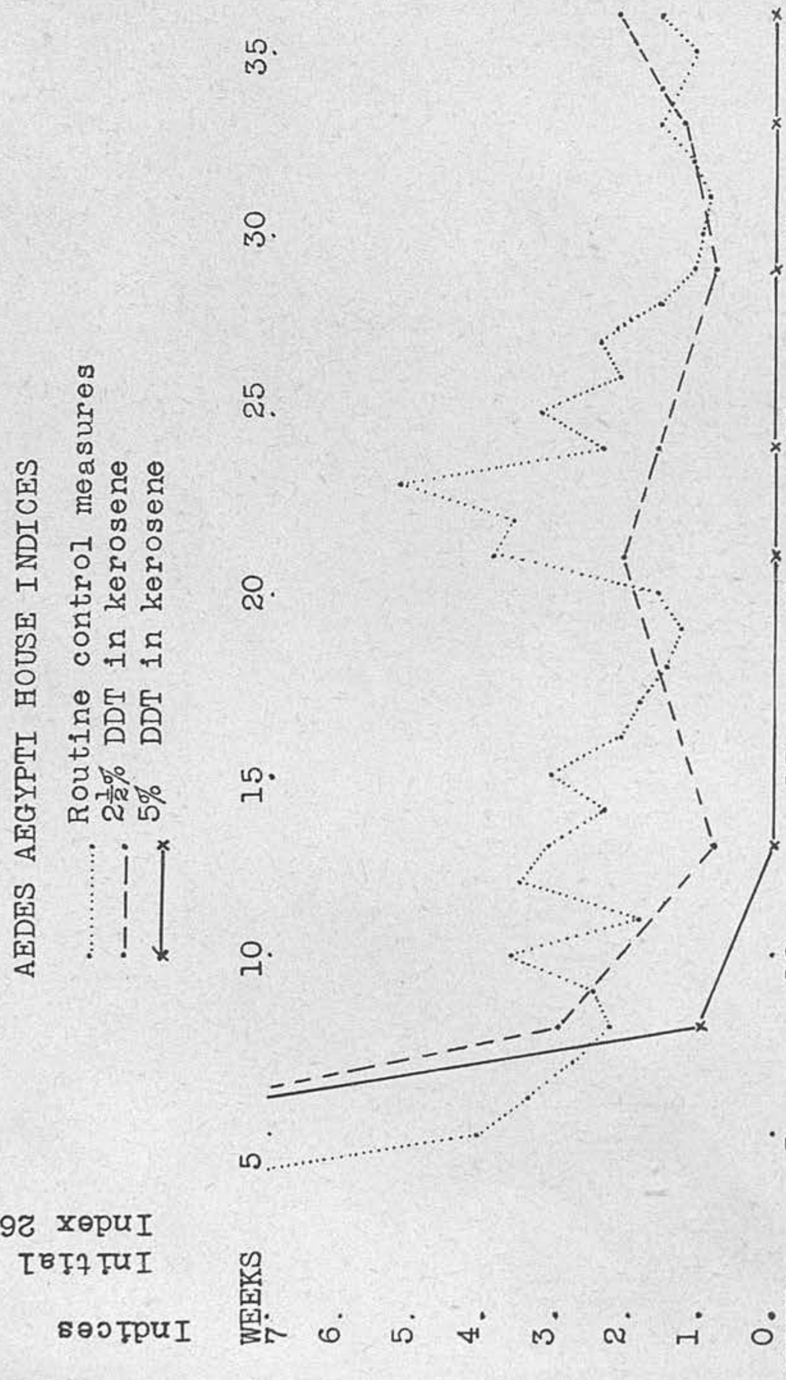

FIGURE 4. Effect of DDT on *Aedes* Breeding.

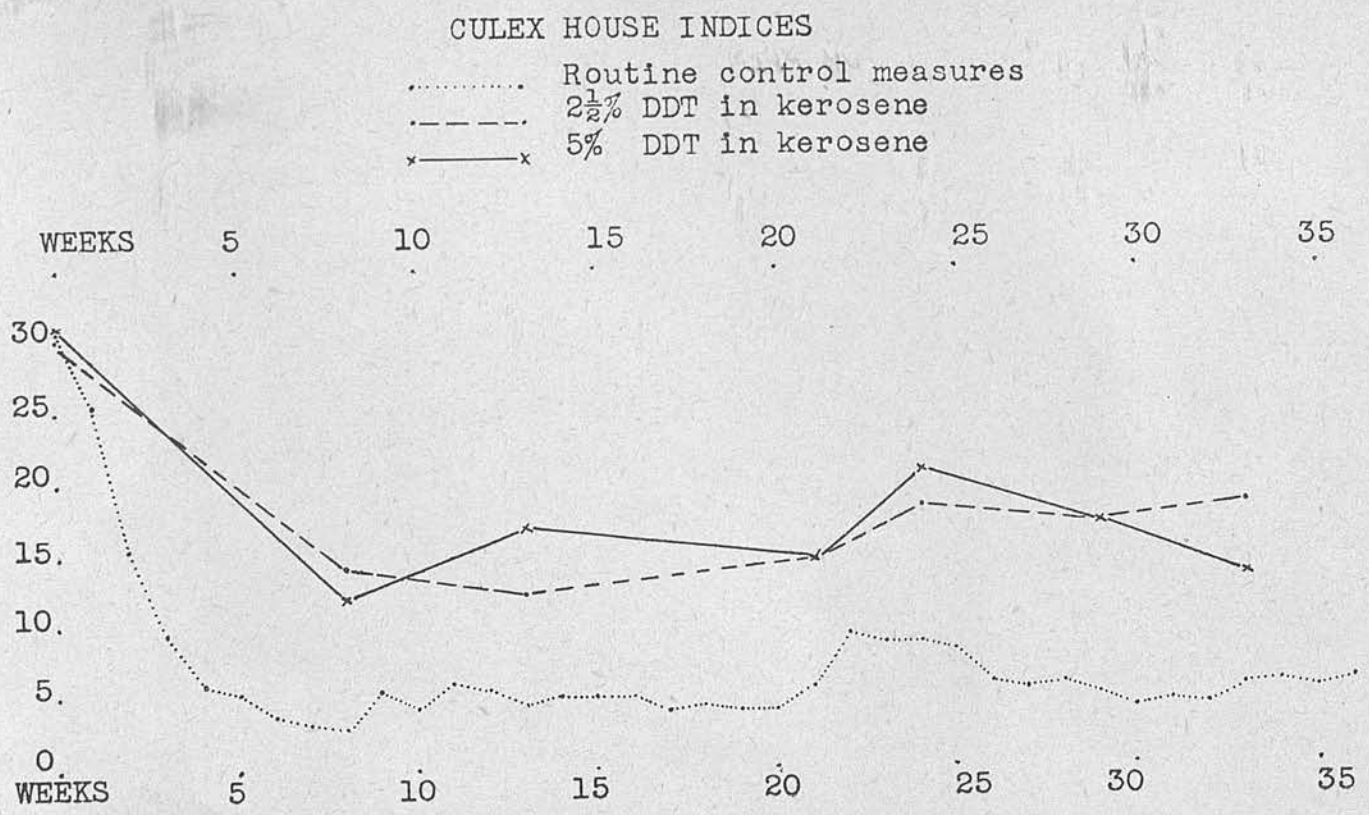

FIGURE 5. Effect of DDT on *Culex* Breeding.

AEDES AEGYPTI CONTROL IN THE ABSENCE OF A PIPED POTABLE WATER SUPPLY

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Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE
Vol. 27, No. 6, November, 1947

Made in United States of America

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In 1939, when British Guiana became a vitally important source of supply of bauxite, the health authorities of the Colony felt that drastic precautions should be taken to protect the air and ocean ports as well as other coastal communities against the introduction of yellow fever. Although yellow fever had not been known to occur in the thickly populated coastal areas of the country since 1909, investigations by Sneath in 1939 (1, 2) established the fact that the jungle form of the disease was present in the interior. An *Aedes aegypti* survey showed that the incidence of these mosquitoes in strategic areas was high.

On the invitation of the Government of the Colony the International Health Division of The Rockefeller Foundation agreed to assist the health authorities in an anti-aegypti campaign. A yellow fever control service was established, and anti-aegypti measures were begun in the fall of 1939. The work was directed first by Dr. A. W. Burke and later by Dr. George Bevier, both of the Foundation staff. I joined the Yellow Fever Control Service in 1942, spent six months in Brazil during 1944 under a Rockefeller Foundation fellowship, studying methods of yellow fever investigation and control, and was appointed chief officer of the Service in December 1945, when Dr. Bevier left British Guiana.

The classical *Aedes aegypti* control measures, as established in Brazil, were employed in British Guiana from 1939 to 1945. Personnel for this work included a staff of zone inspectors to make routine examinations, at 7-day intervals, of all water containers within and in the vicinity of houses, to insure absence of aegypti breeding; a marine inspection service to search for aegypti breeding foci on docks and ships; an adult mosquito capture service; squads to search for hidden foci and a squad to inspect high tanks and water boxes difficult of access. A strict system of supervision and revision was maintained, and it can fairly be claimed that a relatively high standard of efficiency prevailed. In rural areas *A. aegypti* were readily eradicated by the breeding control measures. In urban areas, however, particularly in the city of Georgetown, results were less satisfactory, for although aegypti house indices (*i.e.* the percentage of houses inspected in which adult mosquitoes or water receptacles containing pupae, larvae or eggs were found) became relatively low, these indices fluctuated with the rainfall and eradication was not achieved.

One of the main obstacles to success was the occurrence at times of a 4-day egg-adult cycle. Although such a short cycle was more the exception than the

[1] The British Guiana Yellow Fever Control Service, during 1939–46, was financed jointly by The Rockefeller Foundation, the Government of British Guiana and from a grant under the Colonial Development and Welfare Scheme in the West Indies.

rule, it occurred often enough to permit a certain amount of reinfestation within the 7-day inspection cycle, yet a universal 4-day inspection cycle proved neither practical nor economical. Another deterrent to success was the long delay in legal action against recalcitrant householders who refused to comply with the Yellow Fever Control Regulations. Pending the hearing of a charge against an offender the Yellow Fever Control Service could not take any action against this person.

Late in 1945 a small experiment was carried out to determine the extent to which *A. aegypti* could be controlled by spraying the interiors of houses with DDT. The prolonged residual action of this insecticide proved so effective that routine breeding-control measures were considerably modified during 1946.

The special mosquito-breeding problems encountered in British Guiana and the methods used to overcome them will be described in the following pages.

CONDITIONS CONDUCIVE TO MOSQUITO BREEDING

The city of Georgetown, with approximately 90,000 inhabitants, does not have a piped potable water supply, and for this reason the task of *Aedes aegypti* eradication there has presented great difficulties Rain water is collected off the roofs by galvanized roof-gutters, and the water is led into large storage containers, such as vats, tanks, barrels and drums. Water is drawn from these, as required, and kept in the houses in every imaginable type of usual and unusual vessel. In some areas artesian well water is bought from vendors during the dry seasons and carefully hoarded, being very often hidden away from the prying eyes of inspectors.

Roof-gutters. In 1943 it was recognized that in Georgetown roof-gutters were probably the most dangerous source of mosquito breeding. Eggs laid in these gutters remained viable for months in the dry season and hatched out as soon as rain fell. It is considered that the main "carry over" from one wet season to another was effected in this way. "Flaming" gutters with a blow torch, to destroy eggs, could not be undertaken, as this would have created too serious a fire hazard since the houses in Georgetown are of wooden construction.

The very high temperatures which obtained in the gutters may have been responsible for the fact that the shortest egg-adult cycles were recorded for material from them.

The roof-gutters are often long and badly graded. Rotting fascia boards, to which they are attached, cause sagging. Overhanging trees shed leaves into them, and blockage results. Every conceivable type of refuse, including human and animal excreta, has been found in the gutters of sheds overlooked by windows. In the vertical sections of these gutters, blockage has been caused by cricket and tennis balls, leaves and even a 7-foot snake.

Large squads of men were organized to examine gutters on short inspection cycles. To speed up control work these men cleaned the gutters, did simple grading by means of wooden chocks and, with the householders permission, trimmed overhanging trees. Gutters where breeding was occurring were oiled. While this measure was not always successful in destroying larvae, it stimulated

the householder to take early action so as to avoid having the oil drain into the vat or tank. Notices were served to householders to rectify all major defects. But during the war years there was an acute shortage of materials for repair; and furthermore many householders neglected to make repairs that were possible. Thus a single gutter might remain a potential source of contamination for weeks

FIG. 1. TYPICAL LARGE HOUSE SHOWING ROOF-GUTTERS

FIG. 2. LONG, BADLY GRADED ROOF-GUTTER

on end. In 1946, statutory authority was obtained to perforate defective gutters so that they would not retain water.

Containers for water storage. Large wooden vats or tanks, with an average capacity of about 2,000 gallons, can be found in almost every yard in Georgetown. Sealing of these is too expensive and short lived to be practical, and in order to control mosquito breeding in them five or six "silverbait" (*Tetragonopterus chalceus*) were placed in each one. The squads checked the presence of the larvivorous fish in the vats on each inspection visit, using breadcrumbs to coax them to the surface. Fish die if they are placed in new tanks during the first few weeks after these are put into use, probably because of the presence of toxic

FIG. 3. TREES OVERHANGING ROOF-GUTTERS

FIG. 4. INSPECTING A ROOF-GUTTER WITH A PERISCOPE MIRROR

FIG. 5. CLEANING A ROOF-GUTTER

products from the caulking material. Larvae, however, will live in these new receptacles.

Barrels and drums are widely used as water containers in some areas and, wherever present, they have been a constant source of trouble. They are too small to accommodate fish, which die from the heat. Sealing, which is a relatively simple matter, perishes quickly. A single small flaw in the sealing, often undetected, can cause serious trouble. Many householders delayed sealing such receptacles until they were brought into court—often a slow process—and the

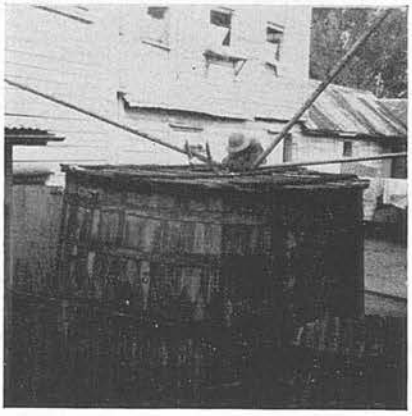

FIG. 6. LARGE WOODEN VAT

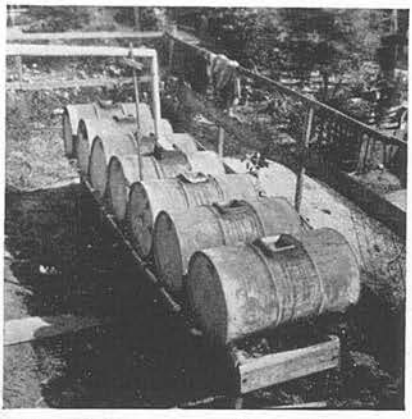

FIG. 7. DRUMS FOR WATER STORAGE

offending barrel and drum would contaminate the surrounding area. Eventually, to save time and money, the Service undertook the sealing of these containers; and although this was bad public health practice it paid large dividends. Drums without covers were marked by the Service, and unauthorised use of them for water storage constituted an offence.

Large zinc-lined water boxes, in which rain water was stored for bathing purposes, presented a similar problem. But since these were not exposed to the weather they could be sealed without fear that the sealing materials would deteriorate quickly.

Wash tubs. Small wooden tubs, for the washing of clothes, proved to be excellent test containers. These vessels cannot be left dry or the wood deteriorates; therefore water is kept in them when they are not in use, and the owner rarely changes the water daily as instructed. The absence of breeding in tubs is an excellent indication of the absence of *Aedes aegypti* on the wing. Oiling these

FIG. 8. WOODEN TUBS FOR WASHING CLOTHES

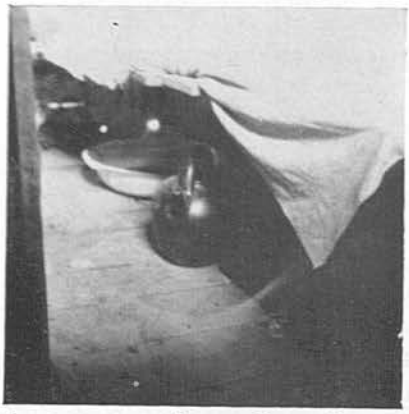

FIG. 9. CLAY CONTAINER AND ENAMEL BASIN HIDDEN UNDER A BED

containers brought forth considerable antagonism, as it requires much effort to render them clean and fit for use again.

Hidden mosquito producing foci. The hoarding of water, as a precious commodity, presents a serious problem in aedes control. The zone inspector has to use considerable tact and ingenuity to make a complete inspection of a house. Frequently water containers have been found hidden under beds and in out-of-the-way places; and more often than not they contained mosquito larvae.

As the *Aedes aegypti* house indices fell, the last few remaining producing foci were almost always of one of three types

(1) Tree holes as high as 30 to 40 feet from the ground. Aedes were often found breeding in very dirty water in these holes, and this was interpreted

as Nature's effort to preserve the species. The holes were either opened up or filled.

(2) Large dirty ground pools under the wooden floors of houses built close to the ground. Here again *Aedes aegypti* was not in its natural habitat, viz. clean water. This type of focus could be eliminated only by ripping up the floor and filling in the pools.

(3) Improperly sealed barrels or drums.

FIG. 10. TREE-HOLES

FIG. 11. UNDER THIS WOODEN FLOOR A LARGE GROUND POOL WAS FOUND

Prevention of Reinfestation

Traffic between infested and clean areas by land, sea and air must be carefully watched. Road traffic did not seem to be a dangerous source of reinfestation, but occasional single female *Aedes aegypti* were, no doubt, imported in this way.

Trains were a serious problem. Pyrethrum spraying at their point of departure for Georgetown reduced, but did not eliminate, the hazard. Treatment with DDT and Gammexane smokes had a brief but not a residual action. Spraying with 5 per cent DDT in kerosene solved the problem. It is advisable to spray all trains and railway stations.

Ocean-going ships rarely brought *Aedes aegypti* into port. Intercolonial schooners, trading between the neighbouring islands and British Guiana, were a constant source of reinfestation of the Georgetown dock area. These vessels were examined in mid-stream, and *Aedes aegypti* adults and breeding foci were eliminated by pyrethrum spraying and oiling of water containers. The Service sealed containers, on request, at the expense of the owner of the schooner.

River craft, except the larger steamers, were not often found to have breeding foci of *Aedes aegypti*. A careful watch over them is nevertheless essential, particularly for maintenance routine.

Discipline and Efficiency

Discipline of inspection squads must be strict. The prompt weeding out of the inefficient and unsuitable inspector is essential if *Aedes aegypti* eradication is to be regarded as a possibility. It has been our unfortunate experience, time and time again, that an unreliable inspector can cost the Service literally thousands of dollars and delay progress for weeks or even months. The process of constantly checking the work of the inspectors, discovering their deficiencies and bringing the inefficient to book, is a most unpleasant one. But it must be done, no matter what control methods are used, if aedes eradication is to be undertaken economically or successfully. Recognition of this fact is regarded as perhaps the most essential feature of an anti-aedes campaign.

Particularly when low indices have been attained, a bonus system, whereby a zone inspector can earn a monthly sum—in addition to, and separate from, his salary—serves as a constant incentive. It produces a higher standard of efficiency in the field and automatically singles out the incompetent inspector. These bonuses are based on efficiency ratings. Inspectors failing to reach a minimum rating on two or more occasions should be discharged. The time comes when a single missed focus will mean a zero rating. This may appear harsh, but it signifies that the inspector failed to find the one focus in his zone during that particular month!

LEGISLATION

The ideal procedure for quick results in aegypti control is the Brazilian system, whereby the officers of the Service deal with offenders directly and fines are collected through the legal department. Where the constant threat of a yellow fever epidemic is not present to stimulate public cooperation, and where the the public has to be educated to accept aedes control, there will always be some persons who will not, or cannot, recognise the need for house inspections and the prompt rectification of any defects in water containers. For the common good, recourse to the law is essential in such cases. A system whereby non-cooperative householders can be promptly dealt with is an urgent necessity, unless much time and money are to be squandered.

Failing such a system the Service will find it cheaper in the long run to undertake the simpler repair jobs, *e.g.* trimming of overhanging trees, simple grading of roof-gutters and the sealing of barrels and drums, as part of the routine work.

DDT AND AEDES CONTROL

We have had experience in aedes control by means of house spraying with DDT in areas where no previous work had been done and in areas where work was in its final stages.

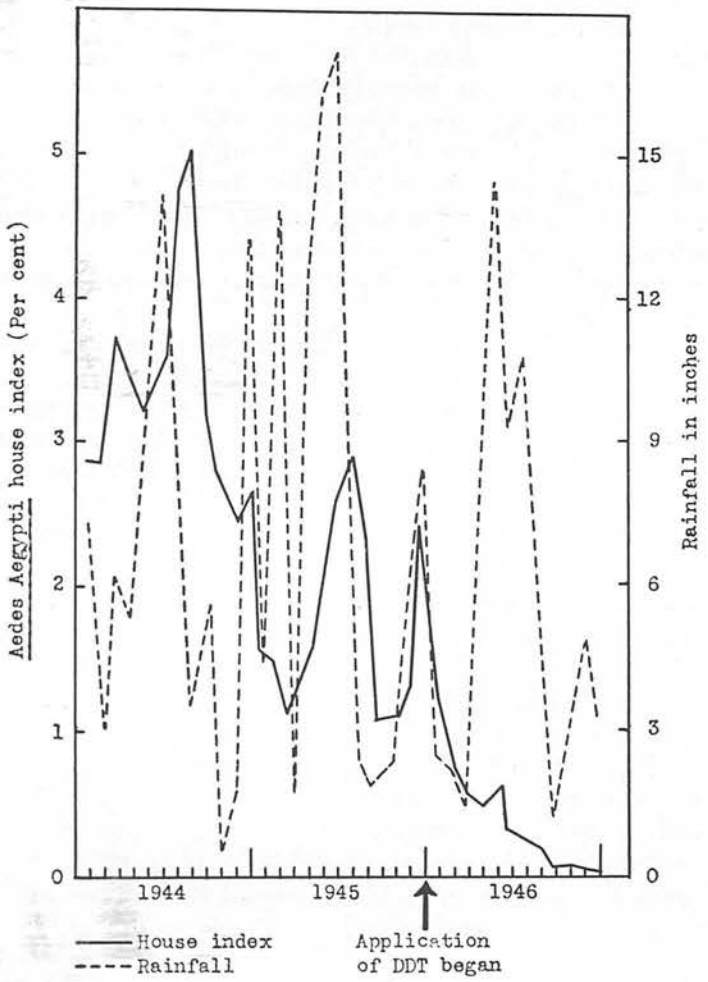

FIG. 12. THE MONTHLY TREND OF AEDES AEGYPTI HOUSE INDICES AND OF RAINFALL BEFORE AND AFTER THE APPLICATION OF DDT, GEORGETOWN, BRITISH GUIANA

A 5 per cent solution of DDT in kerosene—½ lb. technical DDT to 1 gallon commercial non-deodorized kerosene—was applied in an estimated dosage of 100 mgm. DDT per square foot to walls and low ceilings—at a cost of:

DDT	B.W.I. $0.85[2] per lb.
Kerosene	B.W.I. $0.3269 per gallon (both duty free).

[2] Cost is still unstable and varies between B.W.I. $0.75 and B.W.I. $0.95 per lb. U. S. $1.00 is equivalent to B.W.I. $1.19875.

Reprinted from THE AMERICAN JOURNAL OF HYGIENE, Vol. 48, No. 2,
182–190, September, 1948
Printed in U. S. A.

THE RELATIONSHIP OF EXFLAGELLATION IN AVIAN PLASMODIA TO pH AND IMMUNITY IN THE MOSQUITO

BY

DON W. MICKS, P. F. DE CAIRES AND L. B. FRANCO

THE RELATIONSHIP OF EXFLAGELLATION IN AVIAN PLASMODIA TO pH AND IMMUNITY IN THE MOSQUITO[1]

By

DON W. MICKS, P. F. DE CAIRES[2] AND L. B. FRANCO[3]

(Received for publication June 15th, 1948)

Introduction

The process of exflagellation is apparently the earliest known phenomenon associated with the life cycle of the malaria parasite. It was observed by Laveran (1881) at the time of his discovery of the human malaria parasite and it was later described in *Haemoproteus* by Danilewsky (1885), Marchoux (1899) and MacCallum (1898), who discovered the process of fertilization in this same parasite. The latter worker also observed exflagellation and fertilization in *Plasmodium falciparum*. It was not, however, until Ross (1898) noted flagellated organisms in the stomach of the mosquito that the significance of these previous observations was understood.

Subsequent workers became interested in the various factors which influenced the sporogonous cycle. The effect of temperature on the fertilization of *Haemoproteus* was studied by Claus (1903), Wasielewski and Wulkner (1918), Marchoux and Chorine (1932) and others. In general, these investigators found that fertilization could be completed within a rather wide temperature range (8.5 C to 43 C). Kligler and Mer (1937) observed exflagellation of *Plasmodium vivax, P. falciparum* and *P. malariae* at 21 C and somewhat beyond, depending upon the species. Temperatures of 15 to 16 C retarded the process. Marchoux and Chorine (1932) first called attention to the carbon dioxide of the blood as the agent which restrained fertilization in *Haemoproteus,* and Chorine (1933) noted that the process in *P. falciparum* occurred in the presence of saline-citrate but that hydrochloric acid and carbonic acid gas arrested it. He concluded that it was hydrogen-ion concentration which was important.

The present study was undertaken to investigate further the factors determining insusceptibility of certain species of mosquitoes to avian malaria parasites, particularly *Plasmodium elongatum.* Readings of the pH of mosquito stomachs were made in an effort to determine whether or not this factor exerted a major influence on exflagellation and fertilization.

Materials and methods

The mosquito species employed in this study were *Culex pipiens, Culex quinquefasciatus, Aedes aegypti* and *Anopheles quadrimaculatus,* all of which were obtained from insectary colonies. The strains of *P. cathemerium* and *P. relictum* were isolated by one of us (D. M.) from the English sparrow and have been maintained in canaries for the past year. The 5E strain of *P. elongatum* was used and has undergone pas-

[1] From the Department of Parasitology of the School of Hygiene and Public Health of The Johns Hopkins University, Baltimore, Md. The authors wish to express their appreciation to Dr. L. E. Rozeboom, under whose direction this work was carried out, for valuable criticisms and helpful suggestions.

[2] Fellow, International Health Division of the Rockefeller Foundation.

[3] Fellow, United States Public Health Service.

sage through canaries and ducks for several years.

All exflagellation preparations were made and observed at room temperature (21 C to 23 C). Whenever mature gametocytes were sufficiently numerous to assure an abundance of exflagellating forms, preparations were made in the usual manner with a drop of blood from the bird in a drop of isotonic saline-citrate solution. This was immediately covered with a glass coverslip and sealed around the edges with vaseline to prevent excessive evaporation. Preparations were also made directly from the stomach of the mosquito immediately after feeding on infected birds by cutting off the last two abdominal segments and squeezing the blood out on a slide and sealing as previously described. In a few instances, blood films were prepared from the stomach contents at 10-minute intervals from the time of the blood meal for 1.5 hours as a further check on the time of exflagellation and fertilization. These were stained with Giemsa stain.

Preliminary pH readings on mosquito stomachs were obtained colorimetrically with several aqueous indicators covering a total pH range of 5.2 to 7.7. One small drop of the indicator was placed directly on the expressed stomach contents and a Clark color chart (1928) was used for approximation of the pH. These determinations indicated that any differences in readings between mosquito species were in the range below 0.5 of a pH unit and would require more exacting techniques for accurate measurement. Therefore, all pH determinations included herein were obtained with the Beckman pH meter using a 290M micro-electrode in combination with the calomel electrode. The latter was mounted next to the door. The unknown solution was introduced into the upper end of the capillary tube which was filled by capillary attraction and which measured quantities as small as 0.005 ml. The capillary tube was flushed several times with a buffer solution of a known pH and the meter was standardized against this solution and adjusted for asymmetry potential. Prior to undertaking a series of readings, the tube was also flushed several times with saline-citrate solution with a pH of approximately 7.05 and an exact reading was taken. The tube was rinsed with this solution between readings and the pH checked again at the end of the determinations. In order to have sufficient material with which to obtain a single reading on unfed (nonblooded) mosquito stomachs, it was found necessary to dissect out from 2 to 6 and express their contents into the least amount of saline-citrate solution required to fill the tube. The pH of recently fed (blooded) mosquito stomachs was obtained by clipping off the last two abdominal segments and squeezing out a sufficient amount of blood to fill the capillary tube. Since blood clots and extraneous material could easily clog the tube it was found more satisfactory to insert a micropipette into the stomach and draw the blood out into a minute amount of citrated saline before introduction into the tube. In this manner a negligible amount of carbon dioxide was lost due to exposure of the blood to air, and the buffering capacity of the blood counteracted any tendency on the part of the saline-citrate solution to alter the pH reading. The actual rate of loss of carbon dioxide from blood was determined by a series of readings on 2-ml samples of duck blood exposed to the air for several hours in small vials 2 centimeters in diameter. These demonstrated a slow, uniform rise in pH of approximately 0.12 units per hour, re-

gardless of the initial readings which varied from 7.49 to 7.65.

Results

In figure 1 is shown the development of *Plasmodium elongatum* from the intracellular gametocyte stage through fertilization in each of the 4 species of mosquitoes and in a control of bird blood. These curves are based on average figures obtained from data in table 1.

Numerous attempts were made to observe exflagellation of *P. elongatum* in the usual type of preparation with blood from the bird. Occasionally, a few gametocytes rounded up, although numerous observations extending over a period of 7 hours failed to demonstrate any changes in morphology or position of the majority of these forms which continued to remain alive for many hours later. On the other hand, when several *Culex pipiens* were allowed

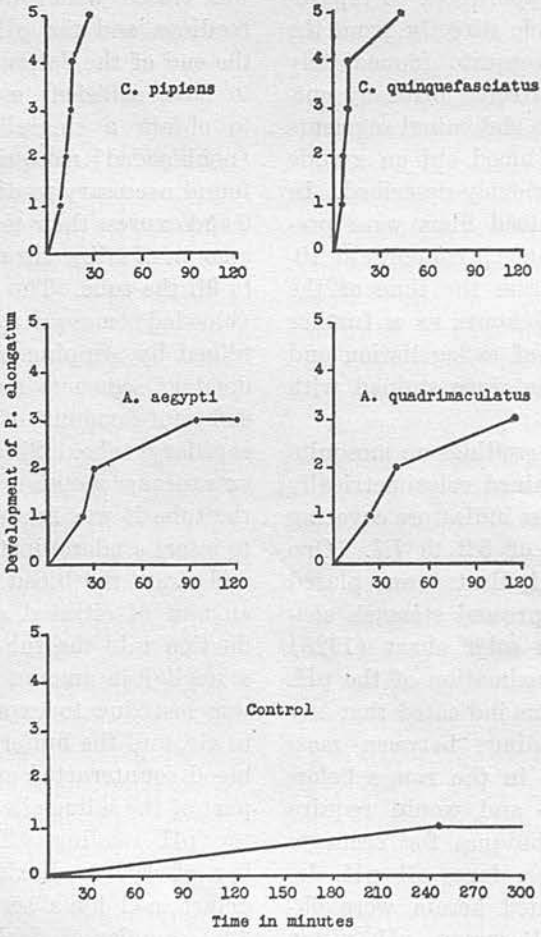

FIGURE 1. The development of *Plasmodium elongatum* is shown from the intracellular gametocyte stage through fertilization in each of the 4 species of mosquitoes and in a control preparation of bird blood. On the perpendicular axis, development stage 1 = gametocytes rounded and extracellular; 2 = active granules; 3 = beginning exflagellation; 4 = exflagellation; and 5 = fertilization.

TABLE 1

Rate of development of P. elongatum *in the stomach cavity of different species of mosquitoes*

Stage of cycle*	*C. pipiens*					*C. quinquefasciatus*					*A. aegypti*					*A. quadrimaculatus*					*Control*				
	Observation no.†					Observation no.					Observation no.					Observation no.					Observation no.				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	5	10	9	7	3	5	4	6	5	4	20	22	30	25	15	25	15	10	18	15	—	—	135	—	360
2	7	12	15	15	5	7	6	7	6	6	30	25	—	32	30	30	30	18	70	50	—	—	—	—	—
3	9	17	22	19	6	9	6	9	6	11	60	32	—	—	190	—	175	—	—	55	—	—	—	—	—
4	10	18	23	20	7	10	7	10	6	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	25	32	35	30	25	36	40	49	56	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Stage 1 = gametocytes rounded and extracellular; 2 = active granules; 3 = beginning exflagellation; 4 = exflagellation; 5 = fertilization.
† Figures indicate time in minutes.

to feed upon a bird with fairly numerous gametocytes and a preparation from the blooded stomach made immediately thereafter, exflagellation was readily observed in as little as 7 minutes. In view of the fact that *Aedes aegypti* and *Anopheles quadrimaculatus* are known to be insusceptible to *P. elongatum,* it was desirable to determine whether development of the parasite proceeded through fertilization in these species. Exflagellation and fertilization were not observed within a period of 2 hours. In most instances essentially all gametocytes were still intracellular at the end of this time; however, exflagellation had apparently begun in a few gametocytes to the extent that one or two microgametes were partially extruded but were in general rather straight and always immobile. Since *Culex quinquefasciatus* has been shown by Huff (1927, 1932) to be insusceptible to infection with *P. elongatum* it is of interest to note that exflagellation and fertilization processes of the parasite occurred very readily in this insusceptible species in an average time of 10 and 43 minutes, respectively.

In order to compare these phenomena in *P. elongatum* with other plasmodia causing avian malaria, *P. relictum* and *P. cathemerium* were utilized in the same manner as previously described, in all 4 species of mosquitoes. In contrast to *P. elongatum,* exflagellation of each of these species was observed in blood taken directly from the bird even though it was often greatly delayed as compared with the speed of the same process in the stomach of the mosquito. In addition to the culicine species, exflagellation of *P. relictum* and *P. cathemerium* occurred very rapidly in *A. quadrimaculatus* and fertilization was observed in the latter parasite. Table

2 compares the average rate of development of the 3 species of parasites in the stomach cavities of various mosquito species.

It became quite apparent that some factor in the stomach of the mosquito must be necessary in initiating the process of exflagellation in *P. elongatum*. In pursuing this point it was found that when stomachs of either *A. aegypti* or *A. quadrimaculatus* were added to blood from the stomach of a recently fed *C. pipiens*, exflagellation was greatly retarded and in some cases not complete. Conversely, when stomachs from unfed (nonblooded) *Culex pipiens* were added to preparations of blooded stomachs of either *A. aegypti* or *A. quadrimaculatus* several gametocytes were seen to round up, with the granules becoming very active, although exflagellation did not continue to completion in any instance.

In view of the fact that previous work has indicated that the hydrogen-ion concentration of the blood may be an important factor influencing exflagellation and fertilization, it seemed worthwhile to pursue this. It was first desirable to know the pH of both canary and duck blood and readings taken on several different birds revealed an average pH of 7.61 for the canary and 7.65 for the duck. Only the duck was used to obtain the pH of blooded stomachs. The results of pH determinations on mosquito stomachs are seen in table 3. Although no significant differences were observed between the readings of unfed stomachs of *C. pipiens, C. quinquefasciatus* and *A. aegypti,* which averaged 7.27, 7.43 and 7.31, respectively, those of *A. quadrimaculatus* stomachs were somewhat higher and averaged 7.75. In making similar determinations on blooded stomachs a rise in pH from 0.16 in *A. quadrimaculatus* to 0.36 in *A. aegypti* was noted; however, with the

TABLE 2

Rate of development of malaria parasites in various species of mosquitoes

Stage of cycle*	*C. pipiens*						*C. quinquefasciatus*						*A. aegypti*						*A. quadrimaculatus*						*Control*					
	elong.†		*cath.*		*rel.*		*elong.*		*cath.*		*rel.*		*elong.*		*cath.*		*rel.*		*elong.*		*cath.*		*rel.*		*elong.*		*cath.*		*rel.*	
	No.‡	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.	No.	M.
1	5	7	4	3	3	5	5	5	4	2	4	3	5	22	5	5	4	16	5	24	3	5	3	12	5	247	5	18	5	23
2	5	11	4	5	3	8	5	6	4	3	4	5	4	29	5	6	44	32	4	40	3	7	3	22	—	—	5	23	5	28
3	5	15	4	8	3	11	5	9	4	3	4	7	3	94	5	8	4	35	2	115	3	7	3	22	—	—	5	32	5	40
4	5	16	4	9	3	18	5	10	4	6	4	8	—	—	5	8	4	38	—	—	3	9	3	24	—	—	5	38	5	45
5	5	27	4	17	3	28	5	43	4	14	4	22	—	—	5	35	4	51	—	—	3	27	—	—	—	—	—	—	—	—

* See footnote * to table 1.

† *Elong.* = *P. elongatum; cath.* = *P. cathemerium; rel.* = *P. relictum.*

‡ Number of observations made with the mean (M.) time (in minutes) required for each stage.

exception of determinations on stomachs of *A. quadrimaculatus* and those of several *A. aegypti*, the maximum pH still remained about the same as or below that of the duck blood.

Our observations confirm those of of Raffaele (1939) that masses of cytoplasm are first pushed out from the microgametocyte and that the gametes are extruded from these masses. It was also noted in many preparations that exflagellation was completed with the microgametocyte remaining attached to the host cell nucleus.

Discussion

The few studies made of the factors governing the susceptibility of mosquitoes to the malaria parasite show them to be very complex. According to Huff (1941) the most critical period for the ookinete is the penetration of the stomach wall and it is here that certain factors, particularly intrinsic ones, operate against penetration by the parasite. He obtained development of *Plasmodium cathemerium* through the ookinete stage (1927) in insusceptible as well as susceptible mosquito species and expressed the opinion (1941) that fertilization and ookinete-formation occurred regardless of whether or not the mosquito is susceptible to infection by the parasite. This has likewise been demonstrated by Nicolaew and Yakolewa (1929) in *Plasmodium vivax* using 3 species of culicine mosquitoes.

In the present study it is of particular interest that the species of mosquitoes used exhibit varying degrees of resistance or immunity to *Plasmodium elongatum* prior to stomach infection. In the stomach of a susceptible species, *Culex pipiens*, exflagellation and fertilization occur with a resulting oocyst infection, while in the stomach of *Culex quinquefasciatus*, an insusceptible species, the former processes occur quite typically but some factor either prevents the penetration of the stomach wall by the ookinete or its development into the oocyst stage. Furthermore, in

Table 3

*pH determinations on unfed (nonblooded) and fed (blooded) mosquito stomachs**

Reading no.	*C. pipiens*				*C. quinquefasciatus*				*A. aegypti*				*A. quadrimaculatus*			
	Unfed		Fed		Unfed		Fed		Unfed		Fed		Unfed		Fed	
	pH	No.	pH	No.	pH	No.	pH	No.	pH	No.	pH	No.	pH	No.	pH	No.
1	7.28	3	7.48	1	7.26	4	7.54	1	7.32	3	7.62	1	7.59	2	7.77	1
2	7.29	3	7.48	1	7.45	4	7.60	1	7.29	6	7.63	1	7.60	2	7.75	1
3	7.27	4	7.45	1	7.49	4	7.59	1	7.32	5	7.63	1	7.58	3	7.76	1
4	7.26	3	7.46	1	7.32	4	7.58	1			7.69	1	7.59	2	7.77	1
5			7.51	1	7.44	4	7.64	1			7.62	1			7.70	1
6			7.45	1	7.48	4	7.59	1			7.70	1			7.78	1
7			7.90	1	7.49	4	7.62	1			7.68	1			7.79	1
8			7.45	1	7.47	4	7.60	1			7.73	1			7.77	1
9			7.43	1	7.48	4	7.58	1			7.73	1			7.75	1
10			7.63	1	7.43	4	7.61	1			7.70	1			7.72	1
Averages	7.27	3	7.52	1	7.43	4	7.60	1	7.31	5	7.67	1	7.59	2	7.75	1

* The number of stomachs used in obtaining each reading is indicated.

the stomachs of either *Aedes aegypti* or *Anopheles quadrimaculatus* the immune response is manifest prior to exflagellation of the gametocyte. Some individual variation in susceptibility was observed in both of these species. In a few preparations exflagellation was able to begin, as judged by the presence of 2 or 3 stationary microgametes, and in one instance, exflagellation of *P. elongatum* in the stomach of an *A. quadrimaculatus* proceeded for almost an hour with very retarded activity and subsequent quiescence with the microgametes still attached. In contrast to *P. elongatum,* all 4 species of mosquitoes were completely susceptible to infection with *P. relictum* and *P. cathemerium* with the single exception of *A. quadrimaculatus* in which fertilization of at least the latter parasite could be completed although this species was completely resistant to stomach infection with all parasites used. Thus, it seems quite clear that in a susceptible species of mosquito there is a definite chemical factor present in the stomach secretions which is responsible for stimulating the parasite to exflagellate and complete fertilization. Likewise, in an insusceptible species, there is a similar factor present which acts in an inhibitory manner, rendering the gametocytes largely inactive. Whether or not this particular chemical factor is also capable of exerting its effect upon the ookinete or whether there exists another factor in the stomach wall is not known but the results of this investigation indicate the possibility of the presence of two chemical factors. Since the addition of stomach contents from unfed *C. pipiens* to infected blood from the bird does not induce exflagellation and in view of the fact that exflagellation may occur in a very few minutes after the gametocytes reach the stomach of the mosquito, it is suggested that the taking of the blood meal is necessary to stimulate certain secretions perhaps enzymatic in nature, which greatly activate the gametocytes to exflagellate and complete fertilization.

Pursuing Chorine's statement (1933) that "it is only the presence of carbonic acid gas which prevents fertilization from taking place," it has been shown with the species of malaria parasites employed here that exflagellation occurs irrespective of the pH of the stomach contents, and that this process may take place at the same or a lower pH than that of the bird blood. Secondly, blood infected with *P. elongatum* may be exposed to the atmosphere for as long as 7 hours with a continuous loss of carbon dioxide and yet the gametocytes do not even approach the exflagellating stage. Finally, the most significant rise in pH from the unfed to the blooded stomach is seen in *A. aegypti* and exflagellation of *P. elongatum* does not occur in this mosquito.

In view of the apparent inhibition of exflagellation of *P. elongatum* in blood from the bird and the fact that both *P. relictum* and *P. cathemerium* may require up to an hour to initiate the process in the same type of preparation, it appears that this method with the strains used is not a satisfactory criterion of the infectiousness of the gametocytes for a mosquito. Even though the sexual forms may be numerous and quite mature in appearance, a recently fed mosquito is the most direct and reliable means of determining the probable infectivity by a given species of parasite. In this same regard Chorine (1933) observed that even though gametocytes of *P. falciparum* may exflagellate after 20 to 25 minutes, it sometimes required 30 to 40 minutes or even longer.

These observations have had one practical laboratory application in that it

was possible to demonstrate to the students all stages of development of the parasite within the mosquito, whereas formerly only exflagellation was observed and infrequently so.

Summary and conclusions

Exflagellation and fertilization of *Plasmodium elongatum* were studied in the stomachs of *Culex pipiens, Culex quinquefasciatus, Aedes aegypti* and *Anopheles quadrimaculatus* and in slide preparations, in an effort to gain new information regarding factors which influence the susceptibility of mosquitoes to a given malarial parasite, particularly *P. elongatum.* These developmental processes of *P. elongatum* were compared with those of *Plasmodium relictum* and *Plasmodium cathemerium* in the same 4 mosquito species. Determinations were made of the pH of unfed (nonblooded) and fed (blooded) mosquito stomachs in order to determine the relationship between exflagellation and pH. The following conclusions may be drawn.

1. In the present study no correlation exists between exflagellation and pH.

2. The immune response of the mosquito can manifest itself sufficiently early to inhibit exflagellation.

3. A particular chemical factor is present in the stomach cavity of *C. pipiens* which greatly activates the gametocytes of *P. elongatum* to exflagellate and complete fertilization.

4. A similar factor, present in the stomachs of *A. aegypti* and *A. quadrimaculatus,* inhibits these processes in the same parasite.

5. The factor which initiates exflagellation of *P. elongatum* in *C. pipiens* appears to be associated with secretions produced by the ingestion of blood, since unfed (nonblooded) stomach contents from the same species do not induce exflagellation upon their addition to infected blood from the bird.

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