

*The Puerto Rico Journal of Public Health*  
and  
*Tropical Medicine*

**GENTIAN VIOLET IN FILARIASIS \***

By B. K. ASHFORD

Of the School of Tropical Medicine of the University of Puerto Rico under the  
auspices of Columbia University,

and

H. McC. SNYDER

Lieutenant-Colonel, M. C. U. S. Army

In 1926, Ernest Carroll Faust, in China, showed that gentian violet was lethal for *Clonorchis sinensis*. Thirty-five milligrams per kilo of body weight reduced infestation from 50 to 100 per cent. C. D. de Langen, in 1928 in Batavia, Dutch East Indies, first used the dye in treating strongyloidiasis. He had 19 cases with hypereosinophilia and constitutional symptoms with scattered foci of bronchopneumonia. He administered gentian violet in doses of from 0.1 to 0.3 gm. three to five times a day and brought about a clinical cure with disappearance of the eosinophilia. Later, Faust (3<sup>b</sup>), in strongyloidiasis without pneumonitis but with gastrointestinal disturbances, reported a disappearance of the organisms from stools and a clinical cure in one week, as a rule, by administering 0.03 gm. oral doses of the dye, three times a day.

In 1902-1903, Ashford (1) made a survey of 250 soldiers of the Puerto Rico Provisional Regiment of Infantry of the United States Army, a mounted battalion in Cayey, and found 12 per cent with microfilariae. In 1932, he felt that a survey of the 65th Regiment of Infantry stationed at San Juan, whose soldiers are native-born Puerto Ricans, would disclose enough carriers of microfilariae to furnish a basis for future investigation on the efficacy of gentian violet in filariasis, as Faust (3<sup>a</sup>) and de Langen (5) have shown it to be entirely harmless to the host in the dose used.

Accordingly, the labor of completing this study was distributed between the two authors who present this paper.

\* Lecture at the School of Tropical Medicine of Puerto Rico, February, 1931.

## I

**The incidence of filarial infestation in the Regiment.**

Between February 15th and 27th, 480 men were examined. Twenty cmm. of blood was spread on slides in a circular area the size of a five-cent piece between the hours of 8:30 and 9:30 p. m. The dried blood was dehemoglobinized by washing in tap water, then fixed in equal parts of alcohol and ether, stained for eight minutes with Bullard's hematoxylin, differentiated by rinsing briefly in 0.5 per cent hydrochloric acid alcohol, washed thereafter for five minutes in tap water, and, after drying, the entire slide was gone over, using a mechanical stage, and the microfilariae were counted.

All cases in which microfilariae were found were counted as above detailed on each seven successive nights in order to strike an average. In all, 18 men were found to contain the larvae in the peripheral blood.

The total number of men examined was divided into three groups according to their residence before enlistment.

Of the 179 previously residing in coast towns, 8 were positive. Four others of the 179 gave histories suspicious of filariasis but only 2 of them were positive for microfilariae.

Of the 146 previously residing in towns and districts of the coastal plain with an elevation of not over 500 feet, 6 were found infested. Five others of the 146 gave histories suggestive of filariasis and only 2 of these showed microfilariae in their peripheral blood.

Of the 155 from mountain towns and districts at an elevation of over 500 feet, but never exceeding 2,500, 4 were positive. Three of the 155 gave histories suspicious of filariasis and in 2, microfilariae were found in the peripheral blood. All of the 4 positive for microfilariae, however, had lived also for longer or shorter periods either on the coast proper or in the slightly elevated coastal districts.

Thus, of 480 presumably healthy soldiers, 18 or 3.7 per cent carried microfilariae in their peripheral blood, and only 9 of these gave a history of having had symptoms which were suggestive of filariasis.

When this is contrasted with the findings in this regiment

in 1902 in which 30 of 250 men, or 12 per cent, were carriers of the embryos, 26 of whom gave histories of previous attacks of probable filariasis, some basis for the popular idea that filariasis has decreased in frequency and severity in Puerto Rico is offered. In fact, of the 1902 series, 18 of 30 men reported having suffered from multiple and seemingly typical attacks and 6 from chyluria. Moreover, over three-fourths of the infested cases had lived the whole or the major part of their lives on the coast or in the coastal plain. To be exact, only 1 of the 4 mountaineers who were positive had seemed to have lived only in the highlands.

In the expedition (4) of the Institute of Tropical Medicine and Hygiene of Puerto Rico to Utuado, a mountain town, in 1913-1914, out of 10,140 cases examined only 5 presented clinical signs and symptoms of filariasis, yet in none could microfilariae be found in the peripheral blood.

Thus we may assume that filariasis in Puerto Rico is almost entirely a disease of the coast proper or the undulating region a few miles back therefrom. This seems worthy of consideration, and we may also feel with gratification that the above data bears out the frequently expressed opinion of medical men and laymen, whose practice and observations were made through the years 1900-1910, that filariasis and elephantiasis have definitely declined in frequency and severity.

## II

### **Standardization of numbers of microfilariae in the peripheral blood and observation and treatment of the carriers in hospital.**

Of the 18 infested, only 17 were available for standardization. The details of this work will be found in Table I. Thirteen of them were now placed in the military hospital and given, *per os*, 0.065 gm. of gentian violet in enteric coated pills three times a day, kindly prepared and furnished by Lilly and Company of Indianapolis, Indiana. These men were given a full diet and were not kept in bed, but their temperature, pulse, and respiration were recorded three times a day, and the total 24-hour urine was examined in two 12-hour daily lots for specific gravity, reaction, color, albumin, sugar,

TABLE I  
MICROFILARIAE IN 20 CMM. OF BLOOD. SEQUENCE OF NIGHTS.

Case	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	Average number of micro-filariae
1 . . . .	8	13	22	18	13	14	5							13.28
2 . . . .	30	37	46	61	63	41	52							47.14
3 . . . .	151	177	173	163	130	171	147							158.85
4 . . . .	6	4	1	1	3	2	1							2.57
5 . . . .	11	21	11	11	11	13	8							12.28
6 . . . .	11	14	8	9	5	7	7							8.71
7 . . . .	66	93	101	118	81	93	98							92.85
8 . . . .	4	9	6	6	6	12	4							6.71
9 . . . .	4		5	4	3	3	4	2	2					3.37
10 . . . .	53		58	40	56	40		17	35					42.71
11 . . . .	6		3	2	3	2	1	1						2.57
12 . . . .	4					3	4	2	2		0	0	0	1.87
13 . . . .	6				5	5	4	2	2	1	4			3.62
14 . . . .	34		28	14	25			26	13	21				23.
15 . . . .	7					2	7	4	2	4	7			4.71
16 . . . .	6					10	15		14		6	6		9.5
17 . . . .	6	7		6	7	4	3		6					5.57

and the sediment for abnormal elements. At no time was any abnormality discovered in the period of days noted in Table II over which these urinalyses continued. The remainder of this table is self-explanatory.

TABLE II  
COURSE OF FIRST THIRTEEN CASES IN HOSPITAL

Case No.	Inclus. dates gentian violet	12-hour urinalysis between these dates	Results	Remarks
1....	3-3-3-26	3-3-3-12	Normal....	Hydrocele and inguinal adenitis when child
2....	3-3-3-26	3-3-3-12	Normal....	Inguinal adenitis when child
3....	2-25-3-22	2-24-3-12	Normal....	Acute filariasis a few months ago, after 20 years service
4....	2-24-3-26	2-24-3-12	Normal....	
5....	2-29-3-26	2-29-3-12	Normal....	
6....	2-25-3-21	2-24-3-12	Normal....	Acute filariasis after 5 years service
7....	2-25-3-25	2-24-3-12	Normal....	
8....	2-19-3-26	2-27-3-12	Normal....	
9....	3-3-3-26	3-2-3-26	Normal....	Operated for inguinal adenitis when child
10....	2-25-3-26	2-24-3-12	Normal....	Acute filariasis 2 years before entering service
11....	2-25-4-5	2-24-3-12	Normal....	Operated for hydrocele when child
12....	3-1-3-26	2-29-3-12	Normal....	
13....	2-24-3-29	2-24-3-12	Normal....	

NOTE: The pulse, temperature and respiration of all of these men was normal during their period of hospitalization.

Cases 3 and 11 were found to have a four plus Wassermann for syphilis; case 5, a plus-minus.

Only one of these cases reported filariasis in his family.

Cases 5, 6 and 7 were found to harbor *Trichuris trichiura* and case 8 had hookworm in the feces.

Of the five remaining cases not placed under treatment in hospital (cases 14, 15, 16, 17 and 18) two reported having had an attack of filariasis previous to enlistment, and one stated that his mother had elephantiasis.

The remaining 5 carriers were left untreated as controls. The hematology of these cases was taken up in July and August, four months later, always between 9 and 12 a.m. and details appear in Table III. The hemoglobin was calculated by a modern calibrated Sahli instrument. Differential counts of leucocytes and measurements of erythrocytes were limited to 100 cells.

It is interesting to note, although the number of cases is too small for nice precision of statement, that Wintrobe's asseveration that "People in these latitudes (New Orleans) have a high normal of 5.5 to 6 million erythrocytes per cmm."

seems to be borne out. It might be added that there seems to be a tendency toward a definite small red cell. The only abnormal feature of these counts is the eosinophile percentage in a majority of cases.

TABLE III  
HEMATOLOGY OF EIGHTEEN MEN WITH MICROFILARIAE

Case No.	Hemoglobin Sahli	Erythrocytes per cmm.	Leucocytes per cmm.	Neutrophiles Per cent	Lymph. Per cent	Mono. Per cent	Eosin. Per cent	Diameter erythrocytes
1.....	85	4,936,000	7,800	69	22	4	5	7.44
2.....	80	6,264,000	12,000	73	17	2	8	7.65
3.....	73	4,016,000	10,200	57	15	4	23	7.65
4.....	100	5,224,000	8,000	64	26	6	4	7.54
5.....	80	4,736,000	11,400	67	12	2	19	.64
6.....	85	5,648,000	9,600	83	9	5	4	7.15
7.....	100	5,860,000	8,600	80	12	5	3	7.96
8.....	95	8,784,000	9,400	58	18	9	15	7.19
9.....	78	5,056,000	10,800	71	14	5	10	7.57
10.....	90	5,372,000	9,200	67	12	6	15	7.63
11.....	75	5,328,000	12,800	74	18	3	5	7.70
12.....	103	6,200,000	8,000	79	10	9	2	7.44
13.....	88	4,944,000	6,200	47	32	3	18	7.18
14.....	89	5,984,000	10,800	62	28	5	5	6.90
15.....	75	4,416,000	9,400	65	28	6	1	7.02
16.....	100	6,144,000	8,400	74	9	3	14	7.19
17.....	80	5,464,000	10,000	68	19	9	4	6.86
18.....	86	4,464,000	9,600	52	28	4	16	7.19
Average..	87.6	5,491,000	9,566	67.16	18.27	5	9.5	7.38

### III

#### Standardization of numbers of microfilariae in the blood of carriers during the administration of gentian violet.

Observations were limited to 13 carriers. The other 5 acted as controls and had no treatment. Details are furnished in Table IV. It is interesting to note that in a very general way, with glaring but not too frequent exceptions, the variation in the number of microfilariae was not great. All specimens were taken at the same hour as before treatment.

### DISCUSSION

It is to O'Connor, Golden and Auchincloss (6) that we owe the very evident opening for a solution of the therapeutic

TABLE IV  
STANDARDIZATION OF CONTENT OF 20 CMM. OF BLOOD IN MICROFILARIAE AFTER  
TREATMENT WAS BEGUN

Date of Examination	Case Number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
2-26			120	2		4							
2-27			286	1		13	36						
2-28			216	5		3	56	3					
2-29			174	2		2	75	3					
3-1			110	2	12		33	4					
3-2			101	1	13	6	80	9		44	1		
3-3			65	0	6	0	43	4		18	1		
3-4	5	19	50	2	10	3	48	1		21	2		
3-5	1	7	98	1	10	0		14	3	13	1	2	8
3-7	1	33	123	0	12	0	54	1	1		0	0	8
3-8	8	37	175	0	17	2	63	11	1	31	0	0	8
3-9	5	17	113	2	6	4	88	1	4	28	2	0	4
3-10	1	21	134	3	14	6	30	5	0	33	0	2	3
3-11	5	20	80	1	10	6	29	12	0	22	1	0	4
3-14	6	25	209	3	11	2	33	10	1	19	4	0	4
3-15	10	15	47	5	12	0	36	9	2	18	0	1	3
3-16	7	19	188	0	10	1	60	7	1	19	0	0	0
3-17	7	20	75	2	12	2	65	10	0	34	0	0	2
3-18	4	13	116	3	6	2	58	7	0	16	1	3	0
3-21	4	10	78	2	3	3	36	3	1	20	0	1	0
3-24	11	31	240		3	3	44	20	1	22	0	0	0
4-1	5	26	128	0	5	6	143	30	2	76	3	1	3
Number of nights.	15	15	22	21	17	4	30	13	0	76	2	1	
						22	21	21	15	17	18	15	14
Average mf. . . .	5.33	20.8	133	1.76	9.94	3.27	54.28	8.42	1.13	30	1	0.73	3.35

problem in filariasis. These investigators proved that the adult worm, *Filaria bancrofti*, does not infallibly choose an obligate residence (although it is often concentrated there), but frequently remains in the vicinity of the mosquito bite in the tissues. This they succeeded in demonstrating by X-ray photographs of dead and calcified worms in the living tissues. Thus, their results condemn to scientific oblivion the formerly well-founded hope that locally injected poisons for the worms and ablation by surgery would settle the question. We are face to face with the need for a drug or substance with the property of the famous 606, a therapeusis meriting the superlative "esterilizans magna". So far, the metals, arsenic, antimony, etc., have failed. Is this quested substance to be an aniline dye?

Gentian violet in the dose employed by us did not cause the disappearance of microfilariae from the peripheral blood of these carriers, but it may well have caused a reduction in their numbers in all save 2 cases (8 and 13). Indeed, it might be worth our while to increase the dose of gentian violet, which in enteric pills does not usually cause, and in our 13 soldier carriers never caused, any untoward symptoms. In fact, intravenous administration may be followed by more definite results than administration *per os*. Carriere and Martin (2) recommend a dose not exceeding 5 to 7 milligrams per kilo of weight. They begin usually with 1 to 5 centigrams in adults of 150 lbs. weight in a variety of affections, but do not mention filariasis.

In the out-patient clinic of the University Hospital of Puerto Rico, in some 50 cases of clinical filariasis and elephantiasis, gentian violet gave most hopeful apparent "results" in prolonging intervals between attacks and, in some instances, in seeming to cure. But the exception proved the rule, for at least in 7 cases, the acute exacerbations recurred, even while taking the dye.

The most that can be hoped is that this small number of cases treated, and possibly other favorable results, may stimulate further experimentation with aniline dyes for the treatment of a disease for which no specific remedy has been found.



TABLE V

(a) COMPARISON OF COUNTS OF MICROFILARIAE IN 20 CMM. OF PERIPHERAL BLOOD BEFORE, DURING AND AFTER THE ADMINISTRATION OF GENTIAN VIOLET

Case	Average 6-8 nights before administration	Average 14-22 nights during administration	Average one night four months after administration	Average seven successive nights six months after administration
1	13.28	5.33	8	Not examined
2	47.14	20.80	51	45.28
3	158.85	133.00	101	88.95
4	2.57	1.76	Not examined	2.57
5	12.28	9.94	3	3.00
6	8.71	3.27	0	Not examined
7	92.85	54.28	53.00	67.42
8	6.71	8.42	5	13.14
9	3.37	1.13	0	0.85
10	42.71	30.00	15	31.42
11	2.57	1.00	2	1.57
12	1.87	0.73	3	1.28
13	3.62	3.35	6	2.55

TABLE V (Continued)

(b) COMPARISON OF COUNTS OF MICROFILARIAE IN 20 CMM. OF PERIPHERAL BLOOD IN FOUR CONTROL CASES TO WHOM GENTIAN VIOLET WAS NOT ADMINISTERED

Case	Average 6-8 nights before administration of gentian violet to first thirteen cases	Average one night four months later	Average seven successive nights six months later
14	23.00	26	31.68
15	4.71	7	5.66
16	9.50	Not examined	22.86
17	5.57	8	9.14

## SUMMARY OF TABLE V

A fall in the number of microfilariae was noted in 11 cases of the 13, but in 2 so slight as to be inconsequential. Of two of the other cases remaining, in one, no change occurred; in the other, a rise. Hence in 9 of the 13 cases a definite reduction in the microfilarial population seems to have occurred, although in two of these it was impossible to reexamine the blood on seven successive nights nine months later, owing to their absence at a great distance at target practice. Whether this reduction was due to the gentian violet administration, however, is an open question. On the contrary, in all of the four controls there was a definite rise in the number of microfilariae.

ACKNOWLEDGMENT

The authors wish to extend their thanks to Colonel Byroade, Commanding Officer, for his courteous assistance and cooperation.

REFERENCES

- (1) ASHFORD, B. K.: (1903) Filariasis in Porto Rico. Medical Record. Nov.
- (2) CARRIERE and MARTIN (1932) Bull. Academie de Medicine. Paris 107: 799.
- (3) FAUST, E. C.: a. (1930) Gentian violet therapy for Strongyloides infection. Editorial. International Med. Digest, 17: 57  
b. (1932) The symptomatology, diagnosis and treatment of Strongyloides infection. J. A. M. A. 98: 2276-2277.
- (4) INSTITUTE OF TROPICAL MEDICINE AND HYGIENE OF PORTO RICO: (1913-1917) Publicaciones e informes del Instituto de Medicina Tropical e Higiene de Porto Rico. Vol. 1. Gov. Printing Office, San Juan, P. R.
- (5) DE LANGEN, C. D.: (1928) Anguillulosis and the syndrome of the "Idiopathic Hypereosinophilia". Med. Dienst. d. Volksgezondheid in Nederl. Indie, 17 (part 4):515.
- (6) O'CONNOR, F. W.; GOLDEN, R. and AUCHINCLOSS, H.: (1931) The Roentgen demonstration of the calcified *Filaria bancrofti* in human tissue. U. S. Naval Bulletin, 29: 17-25.