

A NEW QUANTITATIVE APPROACH TO THE STUDY OF
ANTHELMINTHIC DRUGS, WITH AN EVALUATION OF PIPERAZINE
HEXAHYDRATE, PHTHALYLSULFATHIAZOLE, AND RO 2-5655/3* IN
THE TREATMENT OF ENTEROBIUS INFECTION

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NO UNIFORMITY of opinion exists regarding an effective therapeutic agent for the treatment and control of *Enterobius vermicularis*. Many drugs have been recommended in the literature. However, some have intrinsic defects and inadequacies which prevent them from meeting the requirements for satisfactory therapy and others have not yet been properly evaluated. The present investigation was designed to fill some of the gaps existing in the literature by evaluating the effectiveness of three drugs in the therapy against enterobiasis in man, and by suggesting a method for the quantitative determination of the intensity of infection before and after treatment.

MATERIALS AND METHODS

All of the persons treated for *Enterobius* infections were residents of a mental institution, the Florida Farm Colony, Gainesville, Florida. The patients were housed in buildings of between thirty and forty beds. All the patients in each of eight buildings were investigated. The residents of

six buildings received the three experimental drugs and the residents of two buildings were left untreated, as controls, with the recognition that over a period of time some individuals may become negative without treatment. The patients in each building received the same drug for the same length of time. By treating the entire population in each building we hoped to minimize the possibility of reinfection, of contamination of the samples to be examined, and of errors on the part of the nurse in giving the medication and in collecting the specimens.

Cellulose tape slide preparations were used to collect specimens for evidence of *Enterobius* infection. Preparation and use of the slides were essentially by the technique described by Brooke and co-workers.¹ Only one nurse collected specimens, taking them at night between 9 o'clock and midnight. She washed her hands thoroughly at regular intervals in order to minimize the danger of transferring eggs from tape to tape by contaminated fingers.

In the laboratory, a drop of toluene was placed under the tape and examination was made with a 16 mm. objective and a 5X ocular.² The entire slide was examined, requiring ten to fifteen minutes. This time was greater

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*The use of these drugs does not constitute approval or endorsement by the Public Health Service.

than that required to examine only the portion of the tape that touched the patient's body, as recommended by previous authors. However, preliminary observations indicated that for a significant number of patients only a few eggs in a marginal position were present which might be missed if only a portion of the tape was examined.

Before therapy was begun, the patients were examined daily for six consecutive days. Previous results³ indicate that at least three consecutive examinations are necessary to reveal a fairly constant proportion of the infections observed in populations with different prevalence rates. In evaluating the relative effectiveness of the drugs, another series of six daily consecutive examinations was made, beginning one week after the termination of therapy. This interval of time following therapy was selected in order to preclude the possibility of reinfection since, according to Cram,⁴ approximately fifteen to twenty-eight days are required for the completion of the life cycle of *E. vermicularis*.

From the beginning of this study it was realized that the number of individuals who become worm-free is not the only criterion of effectiveness of a therapeutic program. There have been conflicting reports concerning the anthelmintic activity of various drugs against *E. vermicularis*, primarily because various workers have paid little attention to comparison of the worm burden before and after treatment. In the present work an attempt has been made to obviate these shortcomings by an indirect quantitative determination of the intensity of the infection. With most helminthic infections this can be done by counting the number of eggs found in the feces.

The number of *Enterobius* eggs found in the cellulose tape preparation, however, would obviously have little relationship to the number of worms harbored by a patient, since many variables, such as the peculiar egg-laying habits of the female worms, the time and method of collection, the hygienic habits of the patient, the amount of scratching he does, and various other factors will modify the number of eggs which are picked up by the cellulose tape. However, in view of the fact that egg deposition in the perianal region apparently does not take place every day,⁵ it is believed that the greater the number of worms harbored by a patient, the greater the chances that eggs will be found on the day of collection. Thus, if six consecutive daily specimens are positive in one individual, it can be assumed that the worm burden is higher than if only one or two specimens were positive and the rest were negative. In a given population group, a relative measure of the average intensity of infection can thus be determined by a summation of the product obtained by multiplying the percentage of individuals having positive specimens on no occasion, once, twice, and in progression up to six times by the corresponding factors 0, 1, 2, 3, 4, 5, or 6. The figure thus obtained has been named the coefficient of infection (C.I.) and used for the sake of comparison of the relative intensity of infection in different population groups.

In conference with a consulting physician,* three drugs were selected for testing, piperazine hexahydrate, phthalylsulfathiazole, and RO 2-5655/3 (m-allyloxyphenylear-bamy methyl dipropyl [p-chlorobenzyl] a m m o n i u m

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chloride monohydrate), and were made available free to the institutions by three pharmaceutical companies. The attending physician (C. H. C.) examined each patient, and dosage was determined according to the weight of the individual. During administration of the drugs, the patients were under the careful supervision of the physician and the nurse in attendance in order to detect any toxic reaction resulting from the therapy. Two levels

No obvious toxic symptoms were observed in any of the patients treated with piperazine hexahydrate or phthalylsulfathiazole in either dosage. On the other hand, disagreeable reactions to the RO 2-5655/3 were observed in a number of patients receiving this drug, the following symptoms being noted: diarrhea, nineteen cases; vomiting, ten cases; vomiting, diarrhea, headache, and dizziness in one case. Medication was discon-

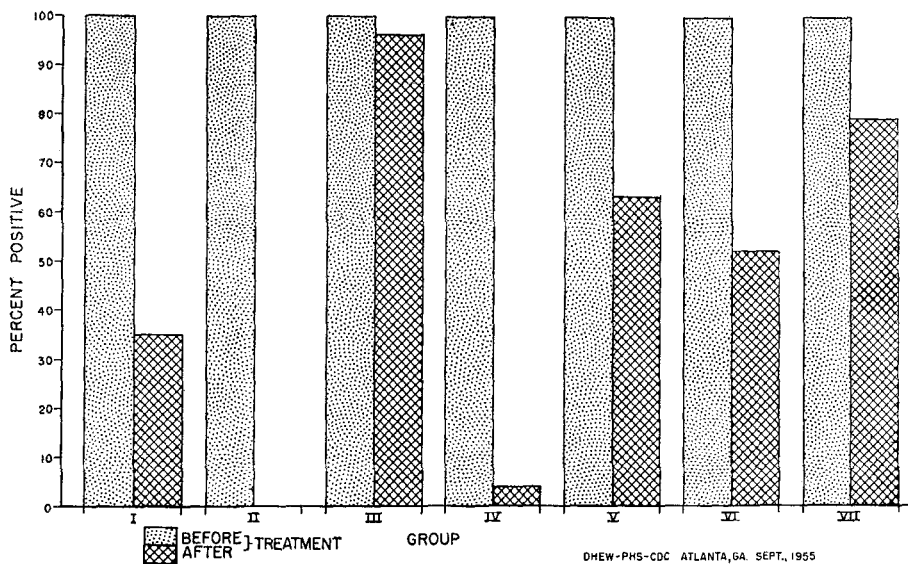


Fig. 1.—A comparison of per cent infection with *E. vermicularis* before and after treatment.

of drugs were administered, one designated as a low dose given for seven consecutive days, the other as a high dose given for fourteen consecutive days. Piperazine hexahydrate was given in the form of a palatable 10 per cent syrup containing 100 mg. per milliliter of syrup; phthalylsulfathiazole was given in an oral suspension containing 1 Gm. of active ingredient per 5 ml. of syrup; and RO 2-5655/3 was administered in the form of 200 mg. tablets. The daily dosages are summarized in Table I.

continued in the latter patient after four days.

Examination of all the cellulose tape preparations could not be completed before the administration of therapy. Therefore, selection of wards on the basis of prevalence and intensity of infection was not possible.

RESULTS AND DISCUSSION

Pretreatment examination in the eight wards included in this experiment revealed that 200 of 265 patients (75 per cent) were infected with *E.*

TABLE I. THE EFFECT OF THERAPY ON THE PER CENT OF INFECTION WITH *E. VERMICULARIS*

GROUP NUMBER	PERSONS IN GROUP	NUMBER INFECTED	PREVALENCE PER CENT	DRUG	DOSAGE, PER DAY	DURATION OF THERAPY IN DAYS	RESULTS OF TREATMENT AMONG INFECTED INDIVIDUALS	
							NUMBER WHO BECAME NEGATIVE	PER CENT WHO BECAME NEGATIVE
I	39	20	51	Piperazine hexahydrate	2 Gm.	7	13	65
II	36	24	67	Piperazine hexahydrate	Under 20 kg., 2 Gm. 20 to 29 kg., 3 Gm. 30 to 39 kg., 4 Gm. 40 kg. and over, 5 Gm.	14	24	100
III	25	24	96	Phthalylsulfathiazole	8 Gm.	7	1	4
IV	33	25	76	Phthalylsulfathiazole	Under 20 kg., 12 Gm. 20 kg. and over, 16 Gm.	14	24	96
V	39	38	97	RO 2-5655/3	2 Gm.	7	14	37
VI	33	27	82	RO 2-5655/3	Under 20 kg., 1.0 Gm. 20 to 29 kg., 1.5 Gm. 30 kg. and over, 2.0 Gm.	14	13	48
VII	60	42	70	None	-	-	9	21

TABLE II. THE EFFECT OF THERAPY ON THE INTENSITY OF INFECTION WITH *E. VERMICULARIS*

GROUP NUMBER	POSITIVES IN GROUP	PER CENT OF PERSONS WITH N POSITIVE SPECIMENS BEFORE TREATMENT						COEFF. OF INFECTION	PER CENT OF PERSONS WITH N POSITIVE SPECIMENS AFTER TREATMENT						COEFF. OF INFECTION
		N=1		N=2		N=3			N=4		N=5		N=6		
		N	%	N	%	N	%		N	%	N	%	N	%	
I	20	30	25	15	20	5	5	260	65	20	0	5	0	5	85
II	24	54	17	13	8	4	4	203	100	0	0	0	0	0	0
III	24	21	8	8	17	4	42	401	4	21	13	8	25	21	354
IV	25	28	36	20	12	4	0	228	96	0	0	4	0	0	12
V	38	21	16	18	16	8	21	337	37	29	18	3	5	3	139
VI	27	30	30	11	22	7	0	246	48	30	7	7	4	4	109
VII	42	35	23	17	10	10	5	252	21	12	12	12	14	12	290

vermicularis. The prevalence rate varied from 51 per cent in one building to 97 per cent in another. No correlation of the prevalence rate with the race and sex of the patients was observed.

The results of the effect of therapy on the per cent of infection are summarized in Table I and presented graphically in Fig. 1.

Piperazine Hexahydrate.—Sixty-five per cent of twenty infected individuals became negative after receiving 2 Gm. of this drug daily for seven days (Group I). When treatment was given for fourteen days in higher doses 100 per cent of twenty-four became negative (Group II).

While piperazine has been used in the past for various purposes, its effectiveness against *E. vermicularis* has only recently been established. White and Standen⁶ and Bumbalo and associates⁷ administered this drug for seven consecutive days, followed by seven days without medication, after which a second course of seven days of therapy was given. Brown and Chan⁸ found that a single course of treatment for fourteen consecutive days was more effective. Essentially similar results were reported by Ricci and Corbo.⁹

Phthalylsulfathiazole.—Whereas only 4 per cent of twenty-four infected individuals became negative following a seven-day course with 8 Gm. daily (Group III), 96 per cent of twenty-five became negative as a result of heavier doses for fourteen consecutive days (Group IV).

Askue and Tufts¹⁰ reported moderate success with this drug and concluded that it is not as effective against *E. vermicularis* as gentian violet.

RO 2-5655/3.—Thirty-seven per cent of thirty-eight individuals became negative after treatment for seven consecutive days with 2 Gm. daily (Group V), whereas 48 per cent of twenty-seven became negative when treated for fourteen consecutive days with somewhat lower doses. Higher dosages could not be tested in view of the toxic manifestations frequently encountered with this drug.

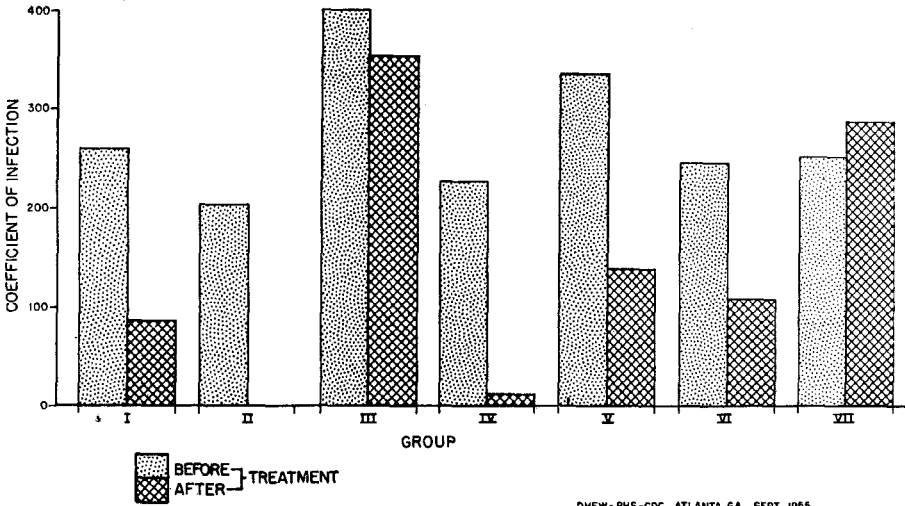
This drug has shown anthelmintic activity against *Syphacia obvelata*, the pinworm of mice,¹¹ and has given encouraging results when used in children at a level of 20 mg. per kilogram of body weight.¹²

Untreated Controls.—Twenty-one per cent of forty-two individuals infected had become negative at the end of the experiment. The chief point of interest to be observed in this group is that if treatment with a wholly ineffective drug had been instituted, apparent cures (on the basis of the criteria used in testing various therapeutic agents) would have resulted in nine instances. The results from the untreated group, in terms of per cent of infection, were not very dissimilar from those obtained with the low dose of phthalylsulfathiazole or with RO 2-5655/3.

The results of the effect of therapy on the intensity of infection are summarized in Table II and presented graphically in Fig. 2. The coefficient of infection before treatment varied from 203 (Group II) to 401 (Group III). A certain correlation between the prevalence of infection in each group and the intensity of infection as determined by the C.I. is apparent. The low dose of piperazine hexahydrate brought a reduction in the C.I. from 260 to 85 (Group I), where-

as the high dose brought about a reduction from 203 to 0 (Group II). With phthalylsulfathiazole, the low dose produced a reduction of the C.I. from 401 to 354 (Group III), whereas the high dose produced a reduction from 228 to 12 (Group IV). RO 2-5655/3 was somewhat effective, although less than the above medications in bringing about a reduction of the C.I. The reduction was approximately the same whether the drug was

brought to light. Whereas a reduction in the per cent of infection was observed among the untreated controls, the opposite was true with regard to the intensity of infection (Group VII). By comparing the results on the persons treated with a low dose of phthalylsulfathiazole (Group III) or with RO 2-5655/3 (Group V and VI) with their controls (Group VII) the anthelmintic activity of these drugs is not apparent on the basis of



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Fig. 2.—A comparison of the intensity of infection with *E. vermicularis* before and after treatment as measured by the coefficient of infection.

administered for seven days (Group V) or for fourteen days (Group VI).

No reduction in the intensity of infection was observed in the untreated control group during the period in which the therapeutic trial was conducted (Group VII). Actually, in the control wards, the C.I. changed from 252 to 290 during the period of observation.

By comparing the results obtained on the per cent infection with those on the intensity of infection (Figs. 1 and 2) several significant facts are

the per cent reduction of infection (Fig. 1), whereas it is evident on the basis of the reduction of the worm burden (Fig. 2). If only the changes from positives to negatives are considered (Fig. 1), the experimental set-up appears to be evenly arranged. Actually, when the C.I. is considered, some irregularities existed among the groups subjected to treatment. As indicated in Fig. 2, the persons treated with low dose of piperazine hexahydrate (Group I) had, on the average, a much lower intensity of infec-

tion than those treated with the low dose of phthalylsulfathiazole (Group III). Although less obviously so, the same relationship existed among those receiving low doses with the various drugs (Groups I, III, and V) and those receiving high doses (Groups II, IV, and VI). In other words, piperazine hexahydrate was not submitted to such a severe test as phthalylsulfathiazole and RO 2-5655/3, and all the three drugs in high doses were not submitted to such a severe test as in the low doses.

indicated in Table III, of the ninety-eight persons who had become negative during the period in which the therapeutic trial was conducted, 40 per cent had been found positive once and only 1 per cent on six examinations. The C.I. for this group was 227. On the other hand, of the 102 patients who remained positive following therapy (failures), 19 per cent had been found positive once and 24 per cent on six examinations. The C.I. in this group was 350. These results are in agreement with and ex-

TABLE III. A COMPARISON OF THE INTENSITY OF INFECTION OF PATIENTS WHO BECAME NEGATIVE DURING THE TIME OF THE THERAPEUTIC TRIAL AND THOSE WHO REMAINED POSITIVE

RESULTS AFTER THERAPEUTIC TRIAL	NUMBER	PER CENT WITH <u>N</u> POSITIVE SPECIMENS BEFORE TREATMENT						COEFFICIENT OF INFECTION
		N = 1	N = 2	N = 3	N = 4	N = 5	N = 6	
Negative	98	40	23	13	19	4	1	227
Positive	102	19	21	16	13	7	24	350

TABLE IV. THE PREVALENCE AND INTENSITY OF INFECTION AMONG PATIENTS RE-EXAMINED 67 DAYS AFTER THERAPY

GROUP NUMBER	EXAMINATION BEFORE THERAPY	PATIENTS RE-EXAMINED 67 DAYS AFTER THERAPY			COEFFICIENT OF INFECTION	
		NUMBER IN GROUP*	NUMBER POSITIVE	PER CENT POSITIVE	BEFORE THERAPY	67 DAYS AFTER THERAPY
II A	Positive	20	4	20	203	65
II B	Negative	10	3	30	0	90
IV A	Positive	24	5	21	337	33
IV B	Negative	8	1	13	0	26

*All the patients in these groups were negative seven through thirteen days after treatment. Their Coefficient of Infection was, therefore, equal to zero.

On the basis of these considerations, it is suggested that the intensity of infection be always considered in evaluating and comparing the anthelmintic activities of various drugs against *E. vermicularis*.

The rate of cure in the various groups seemed to be related to the frequency of positive tests during the initial period of observation or, in other words, to the intensity of infection as measured by the C.I. As

tend the observations reported by Jung and Beaver.⁵

The quantitative determination of the relative intensity of infection could be extended to the examinations of the individual patient in medical practice. By giving a value of 1 to a patient found to be positive only once out of six daily consecutive examinations, 2 to a patient who was positive twice, and so forth up to 6 to a patient who was positive with every

examination, an indirect estimation of the relative worm burden can be made. This estimation can be of value in determining the intensity of infection for clinical and therapeutic purposes. Furthermore, it may throw some light on the degree of egg dissemination in the patient's immediate environment, thus having a bearing on the likelihood of infection among the persons he frequently associates with, primarily the members of his family.

In order to test whether the high proportions of negatives obtained with high dose levels with piperazine hexahydrate (Group II) and with phthalylsulfathiazole (Group IV) represent actual cures or just a temporary sterilization of the worms, the patients were re-examined for six consecutive days beginning with the sixty-seventh day after treatment was discontinued. The results are summarized in Table IV. Since six individuals of Group II left the ward or were transferred during that period, they were not included in the follow-up studies. Also, one patient of Group IV, who remained positive after treatment, is not included in the table. It is of interest to notice, however, that this patient was negative when re-examined for six consecutive days sixty-seven days after treatment. Group II A and Group IV A indicate the persons in wards II and IV who had become negative after treatment. A perusal of Table IV shows that more than two months after treatment only a few individuals in each group had become positive. That these cases represent reinfections and not relapses is evidenced by the fact that no significant difference existed in the Group II A and Group IV A and their respective controls II B and IV B. The C.I. also remained

low with no significant or consistent differences in the various groups.

SUMMARY

Of 265 patients in a mental institution examined for the presence of *Enterobius vermicularis* infection, 200, or 75 per cent, were found to be positive. The prevalence and intensity of infection were determined by six consecutive daily examinations of cellulose tape slide preparations.

A marked reduction in the prevalence and intensity of infection was observed following treatment with relatively high dosages of piperazine hexahydrate or phthalylsulfathiazole for fourteen consecutive days. The effect of treatment with these drugs was still evident more than two months after therapy.

RO 2-5655/3, in the amounts used, was of very limited value in reducing the prevalence of infection. It produced, however, a moderate decrease in the intensity of infection.

Although spontaneous apparent cures were observed in the untreated controls, no marked changes in the intensity of infection occurred during the period of observation. The rate of spontaneous cure in the untreated controls and the degree of effectiveness of the drugs tested were somewhat related to the intensity of infection. These results suggest the use of a coefficient of infection in comparing the therapeutic effectiveness of various drugs.

We are greatly indebted to Dr. Harry Most, Professor of Preventive Medicine, New York University, for his suggestions on the drugs to be used in this investigation. We also wish to express our appreciation to: Burroughs Wellcome & Co., Tuckahoe, New York; Sharp & Dohme, Philadelphia, Pennsyl-

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REFERENCES

1. Brooke, M. M., Donaldson, A. W., and Mitchell, R. B.: A Method of Supplying Cellulose Tape to Physicians for Diagnosis of Enterobiasis, Pub. Health Rep. 64: 897, 1949.
2. Beaver, Paul C.: Methods of Pinworm Diagnosis, Am. J. Trop. Med. 29: 579, 1949.
3. Sadun, E. H., and Melvin, D. M.: 1955. The Probability of Detecting Infections With *Enterobius Vermicularis* by Successive Examinations, J. PEDIAT. 48: 438, 1956.
4. Cram, E. B.: Studies on Oxyuriasis. XXVII. Summary and Conclusions, Am. J. Dis. Child. 65: 45, 1943.
5. Jung, R. C., and Beaver, Paul C.: Treatment of Enterobiasis (Pinworm Infestation) With Diphenan, Egressin and Gentian Violet, Pediatrics 11: 611, 1953.
6. White, R. H. R., and Standen, D. D.: Piperazine in the Treatment of Threadworms in Children, Brit. M. J. 2: 755, 1953.
7. Bumbalo, T. S., Gustina, F. J., and Oleksiak, R. E.: The Treatment of Pinworm Infection (Enterobiasis), J. PEDIAT. 44: 386, 1954.
8. Brown, H. W., and Chan, K. F.: Treatment of *Enterobius vermicularis* Infections With Piperazine, Am. J. Trop. Med. & Hyg. 4: 321, 1955.
9. Ricci, M., and Corbo, S.: Sull'azione dell'idrato di piperazina verso *Enterobius vermicularis*, Riv. parasit. 16: 73, 1955.
10. Askue, W. E., and Tufts, E.: Phthalylsulfathiazole (Sulfathalidine) in the Treatment of Enterobiasis (Pinworm Infection), J. PEDIAT. 44: 380, 1954.
11. Floody, R. J.: Personal communication.
12. Most, H.: Personal communication.

A Letter From Virginia

I now send you the most satisfactory Account I am able, of the dissimilar Birth you have heard me mention with Wonder.

A young Negroe Wench of Col. Mason's began to breed early, and had at the first Birth a Negroe Child. Soon after she was delivered of two, a Mulatto Girl, and a Negroe Boy, named Austin; though born at one Time, they were believed in the Family to be Children of different Fathers, as besides her Negroe Husband, it was well known that the Overseer, a white Man, called Thomas Plum, kept Company with her, to the no small Uneasiness of her black Husband. But the Appearance of the Children was a Proof stronger than any Witness; for Austin was as black a Negroe as I ever saw, and had short, curled, woolly Hair; and in every other Respect was like other Negroes. The Twin Sarah, was as remarkably white, with blue Eyes, and long black Hair, that reached to her Waist. The Wench at the next Birth was delivered of three Children, of which two were Mulattoes, and the other a Negroe.

FROM VIRGINIA GAZETTE (Hunter), Dec. 1, 1752.