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SYSTEMIC TREATMENT OF HUMAN TRICHOMONIASIS WITH A DERIVATIVE OF NITRO-IMIDAZOLE. 8823 R.P.*

BY

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The local treatment of trichomonal vaginitis usually results in cure, but there are the following exceptions:

(1) Some cases are resistant to treatment from the start.

(2) Local treatment of the vagina can have no effect upon infection of the urethra, from which the vagina is re-infected.

(3) In trichomonal urethritis in the male, local methods of treatment up to now have been of little effect, and the logical treatment is systemic.

(4) Very occasionally the *Trichomonas* spreads from its ordinary sites of infection and cannot then be affected by the methods of local treatment currently used.

Some authorities pay little attention to urethritis, believing that normal urine has a trichomonicidal action of its own, and that in men as in women the urethritis tends to undergo spontaneous cure.

This doubtless happens in some cases, but in our experience too infrequently to allow us to count on such a happy outcome. This opinion is shared by numerous authors—Feo, Varano, and Fetter (1956), Harkness and King (1957), Jira (1957), Nicol (1958)—who, though admitting that spontaneous cure occurs in some instances, emphasize the necessity for treatment, and wish that we had more quickly effective methods than we now possess.

Two other points should be borne in mind:

(1) To allow a patient to continue to have a discharge may lead to anxiety and depression.

(2) In many instances trichomoniasis is a venereal infection, and to withhold treatment would be to subject the partner to continued risk of contagion.

For these reasons, we have long been looking for an effective systemic method of treatment similar to the antibiotic treatment of gonorrhoea. Acetarsol, emetine, potassium iodide, mepacrine (Hammer, De Groat, and MacGregor, 1954), and notably Trichomycin and acinitrazole† were studied.

Those last two trichomonicidal substances have been the subject of several publications in the *British Journal of Venereal Diseases*, and of papers presented to the Symposium held at Reims in 1957, and to the Canadian Symposium on Non-Gonococcal Urethritis and Human Trichomoniasis (Montreal, September, 1959). Although their local action is admitted, their systemic action is disputed. Catterall and Nicol (1957) emphasize the fact that neither Trichomycin nor acinitrazole, when given by mouth, confers trichomonicidal activity on the serum or urine.

Because of the criticisms made of these products, one must be cautious in the claims one makes for the action of a new substance. Nevertheless, we are encouraged by our findings in the case of 8823 R.P. for the following reasons:

(1) *Pharmacological.*—Low toxicity, activity *in vitro* and *in vivo*, and trichomonicidal power imparted to the serum and urine.

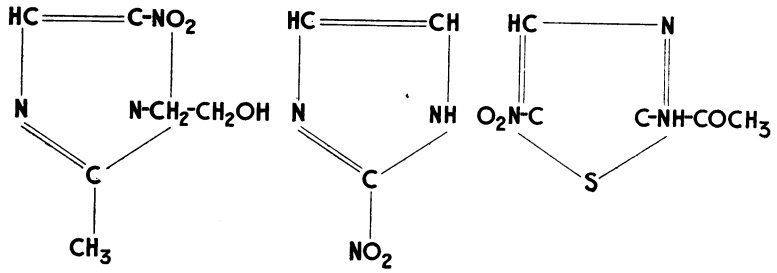
(2) *Clinical.*—Disappearance of the parasite in all male cases and the majority of female cases.

In short, we have been able to study a new chemical compound (kindly put at our disposal by the Specia Laboratories, Paris), which renders the serum and urine trichomonicidal, and which is active both by local and systemic administration. We shall describe our preliminary results, emphasizing that in males treatment was given only by mouth.‡

* Read by Dr. Durel at the General Assembly of the International Union against the Venereal Diseases and Treponematoses, London, October 16th, 1959.

† Generic name for acetamido-2 nitro-5 thiazole in England, France, and Germany. In U.S.A. it is called aminitrozole.

‡ In a few patients a urethral jelly was applied, but in our view the effects of this were negligible.



8823 R.P. AZOMYCIN ACINITRAZOLE

Experimental Study of 8823 R.P.

Horie (1956) described the trichomonicidal properties, in the laboratory, of azomycin (2-nitroimidazole), discovered by Nakamura (1955).

Cosar and Julou (1959), in their search for antibiotics with trichomonicidal activity, confirmed the activity of this substance, and proceeded to study another derivative, more active and less toxic, 1 - β - hydroxyethyl - 2 - methyl - 5 - nitro-imidazole (8823 R.P.).*

The experimental work in which they compared this substance with azomycin and acinitrazole induced us to undertake a clinical study. The chemical formulae of the three products are shown in the Figure.

Toxicity.—Table I indicates toxicity in the mouse after oral administration.

TABLE I

TOXICITY OF 8823 R.P. IN THE MOUSE (COSAR AND JULOU, 1959)

Dosage	8823 R.P. (mg./kg.)	Azomycin	Acinitrazole
L.D.50 (Single Dose) ..	4,300	150	630
Maximum Tolerated Daily Dose (Administered for 5 days)	2,500	50	200

Chronic toxicity tests carried out for one month with the drug given by mouth, show nothing in the rat at a daily dosage level of 50 mg./kg., or in the dog at a daily dosage level of 100 mg./kg.

No functional abnormalities were found and the blood picture was unchanged. The product was well tolerated in the cornea and the skin of the rabbit.

Activity *in vitro*.—The minimum concentrations necessary to destroy 99 per cent. of *T. vaginalis* in 24 hrs are set out in Table II.

* Flagyl R.

TABLE II

IN VITRO ACTIVITY, EXPOSURE FOR 24 HOURS

8823 R.P.	1/400,000	(=2·5 μg./ml.)
Azomycin	1/25,000	(=40 μg./ml.)
Acinitrazole	1/75,000	(=13 μg./ml.)

Activity *in vivo*.—The technique of Lynch, Holley, and Margison (1955) was used. On the day when the *Trichomonas* was injected subcutaneously, and on the four following days, the mice were treated with the drug, and were destroyed on the seventh day. The dosage is shown in Table III.

TABLE III

ORAL DAILY DOSES GIVING COMPLETE PROTECTION (mg./kg. bodyweight) (COSAR AND JULOU, 1959)

8823 R.P.	12·5
Azomycin	50
Acinitrazole	100

Additional Investigations.—8823 R.P. has been shown to have no action on Döderlein's bacillus nor—unfortunately—on *Candida albicans*. We noted in another connexion that it had no immobilizing effect upon the spermatozoa of guinea-pigs or rabbits.

Trichomonicidal Power of Urine or Serum.—We studied the extent to which 8823 R.P. was found in the urine and blood after a single oral dose of 500 mg. (Table IV, opposite). The serum or urine was added to the culture medium, the latter having been inoculated with *Trichomonas* and incubated. Absence of growth of the *Trichomonas* was verified by sub-culture.

Table IV allows us to conclude that 8823 R.P. renders the serum trichomonicidal for at least 3 hours in a dilution of 1/10, and confers the same property on urine for more than 4 hours in dilutions from 1/100 to 1/400.

TABLE IV

TRICHOMONICIDAL POWER AFTER INGESTION OF A SINGLE ORAL DOSE OF 500 mg.

Hours after Ingestion	Patients	Urinary Dilutions				Controls
		1/10	1/100	1/400	1/1000	
0 to 2	R	−/−/− *	+++/−/±	+++	+++/−/+++	+++
	B	−/−/−	±/−/−	−/+++	+++/−/+++	
	D	−	−		++	
2 to 4	R	−/−/−	−/−	−	+/−/++	+++
	B	−/−/−	−/−/−	−/−	+/−/±	
	D	−/−	−		+++	
4 to 6	R	−/−/−	−/−	−/−	++/++	+++
	B	−/−/−	−/−	−/±	++	
6 to 9	R	−/−/−	−	±	+++	+++
	B	−/−/−	−	−	−	
		Serum Dilutions				
		1/10	1/100	1/400	1/1000	
1	R	−	++		++	+++
	D	−	++		++	
2	R	−	++		++	+++
	D	−	++			
3	B	±/−	±/−		−	++

* The results of various tests are separated by oblique lines.

Clinically the drug was used in the form of oral tablets of 250 mg., vaginal tablets of 500 mg. and a 5 per cent. urethral jelly.

We began our trial at the end of 1958, and the early results, both in men and women, were first published in January, 1959.

Clinical Study of 8823 R.P.

Method.—To begin with, we used the drug in all cases of non-gonococcal urethritis, whether *T. vaginalis* was present or not.

(1) *In the Male.*—27 men were treated and *T. vaginalis* was found in thirteen of them (Table V).

TABLE V

RESULTS OF TREATING CASES OF MALE URETHRITIS WITH 8823 R.P. BY MOUTH

Case No.	Duration of Disease	Treatment (mg./day)	Results	Period of Observation (months)
1. (B.341)	6 years	4 to 800 × 7 5 to 800 × 17	Temporary drying up Dried up completely	1
2. (G.393)	7 months	500 × 8	*T. vaginalis* disappeared Discharge persisted	12
3. (B.400)	?	500 × 20	Urethra dry	1½
4. (B.445)	1 year	800 × 10	Urethra dry	1
5. (P.461)	1 year	2 to 500 × 10	Urethra dry	4
6. (N.S.40)	?	500 × 8	Urethra dry	6
7. (H.S.34)	2 years	2 to 500 × 10 + jelly (irritation)	Urethra dry	3
8. (R.S.45)	6 months	2 to 500 × 10 2 to 500 × 10	Relapse Apparent cure	8
9. (B.S.42)	2 years	500 × 8	Urethra dry	6
10. (P.S.30)	1 month	500 × 8	Urethra dry	6
11. (A.S.38)	1 year	2 to 500 × 10	*T. vaginalis* disappeared Still discharge	2
12. (R.S.44)	2 years	2 to 500 × 10	Urethra dry	1
13. (L.491)	10 years	500 to 750 × 10 + jelly on balanitis	Urethra dry	4

These patients had been sent to the Centre for the Study of Non-Gonococcal Urethritis (at the Alfred Fournier Institute), or seen by one of us (A.S.) at the Urological Clinic of the Faculty (Professor B. Fey, Hôpital Cochin), or in private. Most of them were sent to us by urologists, after failure of other forms of treatment. With the exception of two North Africans, all the patients were white. Their ages ranged from 21 to 55 years.

The diagnosis of trichomoniasis was based on the examination of the urethral secretion after May-Grunwald-Giemsa staining, and by culture on the medium devised by one of us (Roiron and Champanhet, 1958). In our view the use of wet preparations is unsatisfactory in males, because in them the parasite shows little mobility. In the tests for cure the same examinations were carried out after prostatic massage and sometimes after centrifuging the urine.

The dosage was usually 500 mg. by mouth daily for 10 consecutive days: one tablet of 250 mg. in the morning and another in the evening. In many cases, on the first day, in order to test the digestive tolerance, the dose was 200 mg. (100 mg. tablets were used for this).

The case histories are all very much the same. Those of the first five patients in the series seen at the Fournier Institute are summarized below:

Case Reports

Case 1.—Recurring attacks of urethritis over the last 6 years. Seen in March, 1958, with an acute urethritis—*T. vaginalis* present. Several antibiotics had been tried. After 7 days ingestion of 8823 R.P., 400 rising to 800 mg. daily, the urethra was dry. Seven days later the urethritis recurred, but no *Trichomonas* could be found. 500 mg. to 800 mg. a day was given for 17 days, when the urethra was again dry. A month later he was still dry, but we have since lost trace of him.

Case 2.—Sub-acute urethritis (*T. vaginalis* present in large numbers) for 7 months. Bemarsal, Conessine, Trichomycin, and Spiramycin had all failed. He was put on 8823 R.P.—500 mg. a day plus jelly locally—and he improved, no *T. vaginalis* being found. During the ensuing 6 months numerous tests were carried out. *T. vaginalis* was never found, but there was intermittent discharge which proved resistant to antibiotics, and on which two further courses of 8823 R.P. had no effect. Urethroscopy and electrocauterization of a focus in the prostate were carried out. Since July, 1958, numerous cultures for *T. vaginalis* have been negative.

Case 3.—Slight urethritis. *T. vaginalis* ++. 8823 R.P. in total daily doses of 500 mg. for 20 days resulted in freedom from discharge for 40 days. No later report.

Case 4.—Recurrent attacks of urethritis for one year, *T. vaginalis* ++; after 10 days treatment with 8823 R.P. in a daily total dose of 800 mg., the urethra was dry, and there were still no signs of urethritis at the end of a month.

Case 5.—After an attack of gonorrhoea, *T. vaginalis* was found in the prostatic secretion for 12 months. Bemarsal and Trichomycin were ineffective. After 10 days treatment with 8823 R.P., in a daily total dose of 200 to 500 mg., there was no discharge, but some slight itching in the urethra. The patient was followed for 4 months, during which time he remained symptom-free.

Our results in the cases of non-trichomonal urethritis are practically negative. We gave 8823 R.P. in doses sometimes reaching as much as 1 g. a day, and treatment was occasionally continued for 20 days. Among fourteen cases where the urethritis was slight or nothing more than a chronic morning drop, there were two that became completely dry, but in view of the indeterminate nature of these cases, we regard this as of no significance.

There were three cases of fungal infection (notably one case of monilial infection in several sites, resistant to Nystatin) and on these 8823 R.P. had no action, as we should have expected from the *in vitro* studies.

(2) *In the Female.*—We have endeavoured to find out if the vaginal secretion, after ingestion of 8823 R.P. had trichomonicidal power. We encountered technical difficulties, but the work is still in progress. Up to the present (except in one doubtful case) we could find no evidence of such trichomonicidal activity, and in our opinion the treatment of trichomonal vaginitis ought to be principally by local administration of the drug, systemic treatment being given, as in the male, where urethritis is also present.

We have prescribed exclusively oral treatment for six women with trichomonal vaginitis. There was one failure, but in the other five patients the results were good. Our aim is to find a method of treating trichomonal vaginitis exclusively by mouth, but we think it premature to speak of having achieved this. In our opinion, in the female, the best results are likely to be obtained by mixed treatment. Our results, after an average of 10 days treatment, were as follows:

(*a*) *Mixed Treatment* — 250 mg. by mouth, morning and evening, and one vaginal tablet of 500 mg. in the evening.

Four cases of non-trichomal vaginitis — Four failures.

21 cases of trichomonal vaginitis — Disappearance of *T. vaginalis* in seventeen (although a discharge persisted in five) and four failures.

(*b*) *Local Treatment Alone*

Four cases of trichomonal vaginitis — Disappearance of *T. vaginitis* in three (although discharge persisted in one), and one failure.

Tolerance

We have given 8823 R.P. by mouth to 27 male and 31 female patients. Two men with non-trichomonal urethritis have taken 1 g. daily for 16 and 18 days without any upset. The side-effects in the other patients are shown in Table VI.

TABLE VI
SIDE-EFFECTS OF ORAL TREATMENT IN 58 PATIENTS

Sex	Male	Female	Total
Some discomfort in the stomach	3	8	11
Including nausea	1	3	4
Diarrhoea	—	1	1
Headache, flushing of the face	—	1	1
Dryness of the mouth	—	2	2

In two female cases treatment was interrupted. It was resumed and well tolerated by one woman, and in the other there was no need for further treatment.

As we were dealing with a nitro-derivative, particular attention was paid to the blood picture. In ten patients (male and female) who had been under treatment for at least 10 days and were examined on the last day of treatment, no abnormality was detected as shown in the findings before and after treatment (Table VII).

TABLE VII
BLOOD PICTURE
(Mean of ten patients)

Blood Cells	Before Treatment	After Treatment
Erythrocytes	4,333,500	4,251,500
Leucocytes	5,358	5,971
Polynuclear neutrophils	65·9 per cent.	64·5 per cent.
eosinophils	3·2 per cent.	2·9 per cent.
basophils	0·8 per cent.	0·8 per cent.
Lymphocytes	22·1 per cent.	24·5 per cent.
Monocytes	8·0 per cent.	7·3 per cent.

In other patients, in whom only one examination was made after treatment, the figures were normal.

Discussion

The failures with substances for which trichomonicidal activity had been claimed in the first published reports call for caution. It is true that we did not use untreated controls or placebos. It is quite possible that the trichomonicidal action of 8823 R.P. may be principally in the urine. A series of only thirteen cases of male urethritis is too small for definite conclusions to be drawn. All the same, our earlier experience in treating such cases, with other forms of treatment, lead us to believe that the *Trichomonas* could not have disappeared spontaneously in a few days in a consecutive series of thirteen cases. The disappearacne of *T. vag.* and the cure of the urethritis seem to be associated with the administration of the drug, and we attribute it to the trichomonicidal power of the serum and urine.

Patients with venereal disease, when they are cured, are often not followed up as well as one would wish, and the period of observation in three cases was no more than a month. All our patients were greatly concerned about their urethritis, for which they had been unsuccessfully treated in the past, and they were pleased with the rapid results obtained with 8823 R.P. In our opinion, these patients would have come back if the urethral discharge had reappeared.

The fact that 8823 R.P. has so far had good effects only where *T. vag.* was present, constitutes additional evidence of the real pathogenicity of the *Trichomonas.*

Results in Canada

In preparation for the first Canadian Symposium on Non-Gonococcal Urethritis, we conveyed the results of our experience with 8823 R.P. to Dr. L. Sylvestre, the President, and to Dr. Z. Gallai, the Secretary of this Symposium. They were kind enough to undertake a study of the drug at Hôpital Notre Dame in Montreal, and the following is a brief resumé of the results reported at the Canadian meeting:

In the Male.—With an average dose of 500 mg. a day for 10 days, Sylvestre, Gallai, and Ethier (1959) obtained fifteen cures in a consecutive series of fifteen cases.

In the Female.—Treatment given was mixed; oral treatment, as in the male, and at the same time, and during the same 10 days, topical treatment with two vaginal tablets each containing 250 mg. In a series of 31 cases, Fortier (1959) reported the disappearance of *T. vag.* and of the discharge in 26 cases: five cases were still under observation at the time of the meeting.

These remarkable results were doubtless attributable to the fact that the Canadian authors were able (as we were not) to treat affected couples simultaneously. It should be added that, as the drug reached them only after some delay, the patients in the interval were kept under observation and

examined every week. During this period of observation the weekly cultures remained positive. Early in the course of treatment they became negative, and continued to be so for periods of observation ranging from 4 to 12 weeks.

We are grateful to our colleagues of Hôpital Notre Dame for the interest they have taken in our preliminary trials, and are glad that they were able to confirm and extend them.

Summary and Conclusion

Systemic treatment of trichomoniasis seems to be logical in the case of the male, and can be regarded as a useful adjunct to local treatment in the female.

Hitherto systemic treatment of trichomoniasis has given very questionable results. It is a problem which has greatly interested us, and we are encouraged by the results obtained with 1-β-hydroxyethyl-2-methyl-5-nitroimidazole (8823 R.P.). The experimental activity of this substance was discovered by Cosar and Julou (1959) and we have demonstrated the trichomonicidal power in the serum and urine after oral administration. In the male the parasite disappeared in all of a series of thirteen consecutive cases.

We must, however, beware of drawing hasty conclusions. We are publishing our results solely in order that our colleagues can subject the drug to thorough investigation. Numerous problems call for wider study, *e.g.* tolerance, effects of exclusively oral treatment in the female, incidence of failures and relapses, and these problems can be solved only by long and patient work.

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Traitement, par voie générale, de la trichomonase à l'aide d'un dérive du nitro-imidazole, 8823 R.P.

Résumé

Le traitement, par la voie générale, de la trichomonase parait le seul logique chez l'homme et peut être consideré comme un très utile complement du traitement vaginal chez la femme.

Jusqu'ici, le traitement général de la trichomonase donnait des resultats discutables. C'est un probleme qui nous a particulièrement interessés et nous retenons avec espoir les resultats que nous avons obtenus avec 1' (hydroxy-2' ethyl)-1 methyl-2 nitro-5 imidazole (8823 R.P.), dont l'action experimentale fût decouverte par Cosar et Julou, dont nous avons constaté le pouvoir trichomonacide sérique et urinaire après ingestion, et qui, chez l'homme, nous a permis de noter la disparition du parasite dans une série continue de 13 cas.