mitral stenosis with cardiac failure attributable to bronchial infection, where any diarrhoea caused by these drugs would be detrimental to the underlying disease.

Summary and Conclusion

Eighty patients with acute respiratory infection of mixed bacterial origin either *per se* or complicating other conditions were treated with chloramphenicol, 2 g. daily for five days. Clinical improvement resulted in 77, three being totally unaffected. Relapse occurred in three patients within one week. The infection was controlled in 73 patients for periods varying from one to eight months after treatment.

Seventy-five patients were quite free from side-effects; dry mouth was observed in three, a mild skin rash in one, and slight transient diarrhoea in one. No blood dyscrasias of any kind were detected.

The potential toxicity of chloramphenicol has been overstressed. If it is given in short courses of 10 g. over five days to patients with acute and severe respiratory infections a satisfactory and prompt response is obtained, with a very low failure and relapse rate, and with negligible toxicity. Chloramphenicol has therefore a definite place in the treatment of such cases, but should not be employed in prolonged dosage or for trivial infections. It may well be that the avoidance of its extensive use in recent years has had the advantage of enabling us to deal effectively with organisms now frequently resistant to the other antibiotics more commonly employed.

We are grateful to Professor D. M. Dunlop for helpful advice and criticism on the preparation of this paper, and for allowing us to study the patients under his care. We also thank Dr. J. H. Bowie and the staff of the bacteriology department of the Royal Infirmary, Edinburgh, for carrying out the sputum cultures and sensitivity tests.

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ORAL TREATMENT OF TRICHOMONAS VAGINITIS WITH AMINITROZOLE

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The work described below is a controlled trial of oral treatment of trichomonas vaginitis, using the oral product aminitrozole (2-acetylaminio-5-nitrothiazole, "tritheon"). During the course of the trial, attempts have been made to culture the *Trichomonas vaginalis*, using the simplified trypticase serum medium of Kupferberg.

Literature

Cuckler, Kupferberg, and Millman (1955) showed that aminitrozole administered orally is effective against trichomonas infection *in vitro* and *in vivo*. It was shown to have a therapeutic effect in experimental vaginal trichomoniasis in monkeys and mice. It is also effective against amoebae and against schistosomiasis. Aminitrozole was well tolerated in six species of animal for extended periods when administered by various routes. In some cases in dogs, brown urine was produced, and this has also been noted in humans.

Perl, Guttmacher, and Raggazoni (1956) treated a series of 174 cases of trichomonas vaginitis with aminitrozole and a cure was obtained in 35%. Out of 48 husbands of infected women, trichomonads were found in the semen of 28 (58%). Of these, 16 who brought further seminal specimens after treatment with aminitrozole were cured after one course of treatment, while a further two were cured after two courses of treatment. The remaining 10 defaulted. In the female trichomonas infection was found to be heaviest one week before and one week after menstruation. When husband and wife both received treatment the percentage of cures was increased.

Plentl, Gray, Neslen, and Dalali (1956) treated a series of cases of trichomonas vaginitis, using aminitrozole in a dose of 150 mg. three times a day. Severe side-effects were noted, including nausea, abdominal pain, anorexia, and dark urine. When the dose was reduced to 100 mg. three times a day, using enteric-coated tablets, side-effects were minimal. Eradication of trichomonas was achieved in 35% and clinical improvement in 67%.

Kupferberg, Johnson, and Sprince (1948) gave a detailed description of the method of preparing a simplified trypticase serum medium for the culture of *Trichomonas vaginalis*. Kean and Day (1954), using this medium for the diagnosis of trichomonas infection, found that culture was more reliable than the hanging drop method for the diagnosis of infection. In 500 consecutive patients examined from a gynaecological clinic the hanging drop was positive in 12.2%, while culture was positive in 18%. They concluded that culture of the organism was essential for diagnosis.

Present Investigation

This investigation was planned with two main objects. The first was to test the effectiveness of aminitroazole for the oral treatment of trichomonas infection. During the course of the trial the second object was to test the use of the simplified trypticase serum medium for the culture of *Trichomonas vaginalis*.

Cases of trichomonas vaginitis were divided alternately into two groups—treated and control. The treated group received aminitroazole 100 mg. three times daily by mouth for ten days, and "aci-jel" vaginal jelly inserted night and morning for three weeks. The control group received aci-jel only, night and morning for three weeks. (Aci-jel is a buffered vaginal jelly with a pH of 4, containing acetic acid, boric acid, oxyquinoline sulphate, ricinoleic acid, and glycerin in a vegetable-gum base.)

In all cases the aim was to take smears at the first visit and after two, four, and six weeks. Treated cases which were negative at six weeks were re-examined at 12 and 24 weeks. Control cases which remained positive after four weeks were given treatment and thus transferred to the treated group. Treated cases positive after six weeks and those which relapsed were given further treatment, some patients receiving three courses in all. Cases regarded as failures with oral treatment were removed from the trial and given local treatment with acetarsol or other forms of medication; in a few cases local symptoms were so persistently severe that it was necessary to institute this after only one course of oral treatment.

The husbands of eight of the patients were examined at St. Paul's Hospital through the kind co-operation of Mr. D. Innes Williams.

Diagnosis

In the early stages of the trial all swabs were submitted to examination of a hanging-drop preparation and to culture using a simplified trypticase serum medium containing antibiotics and prepared as follows: (1) Maltose, 1 g.; (2) bacto agar, 1 g.; (3) distilled water, 400 ml. Heat in autoclave at 10-lb. (0.7 kg. per square cm.) pressure for 10 minutes, dissolve agar. Filter through coarse paper (Reeve Angel). (4) Trypticase (B.B.L.), 20 g.; (5) cysteine hydrochloride, 1.5 g.; (6) 0.5% methylene blue, 0.48 ml.; (7) distilled water to make 600 ml. Adjust pH to 6 with 5N NaOH. Make up to 950 ml. volume with distilled water. Tube in 9.5-ml. amounts. Autoclave at 15-lb. (1.05 kg. per square cm.) pressure for 15 minutes and cool to room temperature. Add 0.5 ml. of sterile serum 100%, which has been adjusted to pH 6. Add 250 units of penicillin and 1 mg. of streptomycin per ml. of medium. (In practice 5-ml. aliquots had been found satisfactory.)

In the early stages of the trial a swab of the vaginal secretion was rotated in 1–2 ml. of saline, brought at once to the laboratory, and immediately examined. Several drops of the suspension were then inoculated into bijou bottles containing 5 ml. of the culture medium. The bottles were incubated at 37° C. and the cultures examined after 48 and 96 hours. As the results were unsatisfactory the technique was modified, the swab being placed immediately into the culture medium to avoid the addition of saline and ensure a heavy inoculum.

The temperature of the incubator was next tested and found to vary by several degrees on different shelves. Stabilization at 37° C. was attained, but the results remained unconvincing.

About 40% of those preparations which revealed large numbers of trichomonads in the hanging drop were still positive after 48 hours' cultivation, but in most instances the numbers were much reduced, motility was sluggish, and disintegrating forms were visible. If the wet preparation showed only a few trichomonads, survival after 48 hours was infrequent, and after 96 hours rare. The impression gained was that the medium encouraged the protozoa to survive for a limited period but did not promote their growth.

On only one occasion was a positive culture obtained from a negative hanging-drop preparation, although several of these strongly suggested a trichomonas infection. Latterly it was felt that thorough examination of a hanging-drop preparation was the far more reliable guide to the presence of trichomonads and culturing was discontinued.

The results obtained from 113 swabs examined directly, after 48 and 96 hours' culture, are shown in Table I. These results were disappointing, and compared very unfavourably with those reported by American workers using a similar medium.

TABLE I

	Hanging Drop Positive (77)		Hanging Drop Negative (36)	
	Positive	Negative	Positive	Negative
48-hour culture ..	30	47	1	35
96- " " ..	10	67	0	36

Results

A total of 60 women were enrolled in the trial, consisting initially of 34 treated cases and 26 controls; 9 in the treated group and 5 in the control group defaulted and did not attend for further treatment. The results in the remainder are shown in Table II.

TABLE II

Treated Group (25)	
Cured of trichomonas vaginitis ..	5
but monilial infection appeared ..	1
Still positive after treatment ..	11
Relapsed after one negative swab or more ..	6
Underwent hysterectomy, cured of trichomonas vaginitis ..	1
Toxic symptoms; did not take tablets ..	1
Control Group (21)	
Remained positive, treated ..	17
Dilatation and curettage, and cauterization of cervix, cured ..	1
Symptomatic improvement ..	2
Other treatment given ..	1
Controls Treated with Aminitroazole (17)	
Persistently positive ..	12
Relapse ..	2
Defaulted ..	1
Recent cases not followed up ..	2

Twelve of the 25 treated patients had one course of aminitroazole, six had two courses, and seven had three courses. Of the five cured of trichomonas vaginitis, four had one course of aminitroazole and one had two courses.

Eight of the treated controls had one course of aminitroazole, four had two courses, and two had three courses.

Thus of 37 patients (23 treated and 14 treated controls) who received treatment with aminitroazole and aci-jel, six were cured of trichomonas vaginitis.

Husbands Examined by Prostatic Smear (St. Paul's Hospital).—Eight husbands were examined: five were negative, two were positive, and one had pus but no trichomonads. The two men with positive smears were both treated and the smears became negative; the wife of one was treated but relapsed after 18 weeks; the wife of the other was cured after treatment with aminitroazole. In the man with pus only, this disappeared after treatment with aminitroazole, and his wife was cured by a further course of aminitroazole.

Summary and Conclusions

Aminitroazole (2-acetyl-amino-5-nitrothiazole, "tritheon") has been recommended for the oral treatment of trichomonas vaginitis.

The results of a controlled trial of oral treatment are presented.

Attempts to culture *Trichomonas vaginalis*, using a simplified trypticase serum medium, have proved unsuccessful; careful examination of a hanging-drop preparation has proved the best method of diagnosis.

The main conclusion of this work has been that aminitroazole with a buffered acid vaginal jelly ("aci-jel") cannot be recommended for the treatment of trichomonas vaginitis. Only 6 out of 37 patients treated

were cured, and in fact the results have proved so disappointing that it has been reluctantly decided to abandon the trial.

Further work might show whether aminotroazole is effective in eliminating trichomonads in the female urinary tract and in the male, but the results so far suggest that it is not suitable alone for vaginal infections. There may be a place for it in conjunction with an effective trichomonicide, used locally in the vagina, especially in chronic cases, but its use without such treatment does not give sufficiently good results to make it a practical method.

We acknowledge our indebtedness to Ortho Pharmaceutical Limited for their generous support of this investigation. This has included help with the technical side and supplies of tritheon, aci-jel, and materials for the culture medium. This help has been given without prejudice to any results that might be obtained. We also thank Mr. D. Innes Williams and the staff of St. Paul's Hospital. Miss Landau, Miss Mocatta, and Miss Hurter kindly allowed us to treat patients under their care.

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SENSITIVITY TO FOLIC ACID

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Folic acid (pteroylglutamic acid) has been extensively used since 1945 not only for the treatment of certain megaloblastic anaemias but also as an ingredient of numerous vitamin preparations. In spite of this wide use the only report of sensitivity to this drug is that of Mitchell, Vilter, and Vilter (1949). Their patient, a woman aged 35, reacted on two occasions to folic acid. On the first occasion she developed a pruritic rash which disappeared on withdrawal of the drug; on the second occasion an anaphylactoid reaction followed an intravenous dose. Subsequent skin tests confirmed that she was sensitive to folic acid. She was known also to be sensitive to phenobarbitone, various foods, wool, soaps, and face-powder.

The purpose of this paper is to report an example of sensitivity to folic acid arising in a previously healthy subject. The results of intradermal tests with folic acid and closely related compounds are also described.

Case Report

A healthy male doctor aged 35 was given, for experimental purposes, 3 mg. of folic acid as a solution of the sodium salt by mouth without untoward reaction. The material was absorbed normally, for the serum folic-acid concentrations, measured with *Streptococcus faecalis* R assay (Jukes, 1955), rose from 0 to 40 μ g. per ml. in the first four hours after the dose.

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Six weeks later the same subject was given four 5-mg. tablets of folic acid by mouth. Forty minutes after taking the tablets he complained of general malaise, aching pain in the lower thoracic region, and respiratory difficulty with restricted inspiration. This was followed 20 minutes later by itching of the palms of the hands and soles of the feet. Pruritus then became generalized; it was worse at the flexures, and was associated with a generalized erythematous rash, most pronounced on the thighs and forearms. The reactions disappeared within three hours of taking the folic acid.

Investigations.—Intradermal tests were carried out by injecting 0.1 ml. of the test solutions into the skin on the front of the forearm. A test solution of folic acid was included in each set of tests together with a distilled-water control. Positive reactions with weal, red flare, and itching were obvious in 10 to 15 minutes after the injection; they reached a peak in about 20 minutes and disappeared in 45 to 60 minutes. The substances tested, the concentrations of test solutions, and the results are given in the Table.

Two different samples of commercially available folic acid (pteroylglutamic acid) gave positive reactions after intradermal injection in concentrations of 5 μ g. per ml. or more, as did similar concentrations of a crystalline preparation of folic acid of high (97%) purity kindly supplied by Dr. William L. Williams, of Lederle Laboratories Inc. Similar reactions followed the intradermal injection of the same concentrations of aminopterin (4-aminopteroylglutamic acid). On the other hand, no reactions followed the intradermal injection of the following solutions: two preparations of folinic acid ("leucovorin," N⁵-formyltetrahydropteroylglutamic acid), N¹⁰-formyltetrahydropteroylglutamic acid; anhydroleucovorin; a folic acid conjugate "teropterin"; and amethopterin (4-amino-N¹⁰-methylpteroylglutamic acid).

Free antibody was tested for by a precipitation method using a constant antibody optimal-proportions technique (Dean and Webb, 1926) and also by adsorbing folic acid to tanned red cells and testing the ability of the patient's serum to agglutinate these cells (Boyden, 1951). No antibodies were demonstrated by either method.

Six months after the development of sensitivity the blood count was: red cells, 5,300,000 per c.mm.; haemoglobin, 15.4 g. per 100 ml.; packed cell volume, 46%; white cells, 8,000 per c.mm. There were no abnormalities in the stained film.

Discussion

The results of skin tests in our patient show that while he was sensitive to folic acid (pteroylglutamic acid) and its analogue aminopterin (4-aminopteroylglutamic acid), he did not react to folinic acid, anhydroleucovorin, N¹⁰-formyltetrahydropteroylglutamic acid, and amethopterin, which are related compounds with substituents in the 5 and 10 positions (see Table).

None of the compounds we used was absolutely pure; the purest folic acid obtainable contained about 3% impurities, and the aminopterin and related compounds contained traces of folic acid as well as other chemical contaminants.

Results of Intradermal Tests with Folic Acid and Related Compounds

Substance	Concentration (μ g. per ml.)	Result
Commercial folic acid (a) ..	{ < 5 5 to 100	— +
" " " (b) ..	{ < 5 5 to 100	— +
Purified " " " ..	{ < 5 5 to 100	— +
Aminopterin	{ < 5 5 to 100	— +
Folinic acid (a) (leucovorin) ..	250	—
Folinic acid (b)	250	—
Anhydroleucovorin	Saturated solution	—
N ¹⁰ -formyltetrahydropteroylglutamic acid	250	—
Teropterin	250	—
Amethopterin	250	—