FURTHER OBSERVATIONS ON STRAIN SENSITIVITY OF *Trichomonas vaginalis* TO METRONIDAZOLE*

BY

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Squires and McFadzean (1962) reviewed the question of strain sensitivity of *Trichomonas vaginalis* to metronidazole. Since then examinations of the sensitivities of strains isolated from occasional patients who had apparently not responded to treatment with metronidazole have been continued. The results of these examinations are described.

It was also thought worth while to examine the strain sensitivities of random cultures of the parasite lest there had been a change which was not as yet reflected by therapeutic failures. At the same time, in view of the observation by Nicol, Evans, McFadzean, and Squires (1966) that organisms of the genus *Mimae* could "inactivate" the drug, the vaginal flora of these patients were also examined for the presence of organisms capable of inactivating the compound.

Methods

The techniques for isolation and sensitivity testing of the parasites in the case of patients who had apparently failed to respond to treatment were as described by Squires and McFadzean (1962). In the case of the random samples, which came from the London Hospital, the Middlesex Hospital, and St. Thomas' Hospital, the initial media used for isolating the organisms did not contain antibiotics. Subsequent subcultures were made into medium containing antibiotics before determining the sensitivities of the trichomonads to metronidazole. The initial cultures were also plated out on blood agar. The bacteria were subsequently inoculated into tryptose phosphate medium containing 10 and/or 100 µg./ml. metronidazole. After incubation for 24 hours at 37°C. the tubes were centrifuged at 4,000 r.p.m. for 10 minutes, the supernatant was removed and was sterilized by being held at 100°C. for 20 minutes, and the metronidazole content of the supernatant was estimated polarographically. These procedures were undertaken initially with the mixed flora present. If inactivation occurred, the bacterial species were then separated, identified where possible, and tested individually for their abilities to inactivate the compound.

Results

45 cultures were submitted from different parts of Great Britain over the period 1962 to 1968, from patients who had apparently not responded to treatment with metronidazole. Trichomonads were isolated and cultured from 25 (56 per cent.) of these and all were found to be sensitive to 0.5 to 1 µg./ml. metronidazole. Trichomonads were not grown from twenty samples, and in eleven of these there was no evidence of trichomonads having been inoculated as shown by the absence of dead organisms in centrifuged deposits of the cultures.

22 of the specimens were also examined for the presence of organisms capable of inactivating metronidazole. Of these, eleven (50 per cent.) had organisms present which inactivated the drug, as assessed polarographically. In these instances the organisms were not identified. In some cases, however, where no organisms capable of inactivation were found, it was shown that the absorption of metronidazole, as judged by serum concentrations, was poor.

84 random inocula were made from patients with trichomonal vaginitis attending outpatient clinics of three London hospitals; *T. vaginalis* was grown from 55 (65 per cent.) of these and in all instances the sensitivities were within the normal range.

56 (67 per cent.) of the 84 inocula contained bacteria. These were not directly related to those from which *T. vaginalis* was grown. Sixteen out of the 56 (28 per cent.) were capable of inactivating metronidazole (see Table): it so happened that no one patient harboured more than one organism capable of inactivating the compound. Seven of the isolates were *Strep. faecalis*, four were *E. coli*, three were Gram-positive cocci, one was a *Proteus* sp., and one a *Klebsiella* sp. It was possible to follow-up eleven of the sixteen patients. Of these, five responded satisfactorily to 200 mg. metronidazole three times a day for 7 days and five defaulted. One patient had three cervical smears positive for

*Received for publication October 8, 1968.

TABLE
DETAILS OF THE RANDOM SAMPLES WHICH CONTAINED MICRO-ORGANISMS CAPABLE OF
INACTIVATING METRONIDAZOLE

Isolate No.	Contaminating Organism			Response to Treatment with Metronidazole
	Type	Percentage Inactivation of Metronidazole		
		10 µg./ml.	100 µg./ml.	
ST 7	Gram-positive cocci	100	60	3 cervical smears positive for <i>T. vaginalis</i> after treatment
ST 9	<i>E. coli</i>	65	60	Defaulted
M 5	<i>Strep. faecalis</i>	—	70	Defaulted
M 11	Gram-positive cocci	100	100	Satisfactory
M 15	<i>E. coli</i>	100	40	Defaulted
M 16	<i>Strep. faecalis</i>	100	70	Defaulted
M 17	<i>Strep. faecalis</i>	100	65	Satisfactory
M 19	<i>Strep. faecalis</i>	100	70	Satisfactory
M 31	<i>Proteus sp.</i>	—	45	Satisfactory
M 38	<i>Klebsiella sp.</i>	—	40	Defaulted
M 40	<i>Strep. faecalis</i>	100	40	Satisfactory
ST 1	<i>E. coli</i>	100	60	Unknown
M 23	<i>Strep. faecalis</i>	100	75	Unknown
M 34	Gram-positive cocci	—	70	Unknown
M 49	<i>Strep. faecalis</i>	—	30	Unknown
M 52	<i>E. coli</i>	—	40	Unknown

T. vaginalis after the standard treatment with metronidazole.

Discussion

There is still no evidence of the development of resistance of the trichomonad to metronidazole after widespread use of the compound in this country for some 8 years.

The question of the importance of the presence in the vagina of organisms capable of inactivating metronidazole has yet to be resolved. One patient had trichomonads present in cervical smears after treatment, but the reliability of identification of the parasite in the smears is in doubt (Hill, 1968).

Summary

The sensitivities to metronidazole of 25 strains of *T. vaginalis* isolated from patients who had apparently failed to respond to treatment with metronidazole, and the sensitivities of 55 isolates obtained at random, showed no evidence of the development of resistance to this compound.

The importance of the presence of bacteria in the vagina capable of inactivating the compound is, as yet, undetermined.

We are grateful to Dr R. D. Catterall, of the Middlesex Hospital, Dr C. S. Nicol, of St. Thomas' Hospital, and Dr P. Rodin, of the Whitechapel Clinic, the London Hospital, for kindly submitting cultures of trichomonads from their patients. We are also grateful to Mr M. J. Parnell for the polarographic estimations.

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Des observations additionnelles faites au sujet de la sensibilité du *T. vaginalis* au métronidazole

RÉSUMÉ

La sensibilité au métronidazole de 25 souches de *T. vaginalis* isolées de malades qui n'avaient pas apparemment répondu au traitement par le métronidazole, et la sensibilité de 55 souches obtenues au hasard ne montrait aucun signe de développement d'une résistance à ce médicament.

L'importance de la présence de bactéries dans le vagin capables de rendre ce médicament inactif reste jusqu'ici non établie.