ABSORPTION AND ELIMINATION OF GRISEOFULVIN FROM THE ALIMENTARY TRACT OF THE RAT

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A new method is described for determining griseofulvin in the alimentary canal of rats dosed orally with the antibiotic: slightly modified, the method was used for measuring faecal griseofulvin. Attempts have been made to correlate the appearance of griseofulvin in the blood stream with its disappearance from the alimentary canal and to assess how much of a single oral dose is absorbed. Lack of griseofulvin at its main absorption sites in the alimentary tract was not responsible for the decline in blood level 4 hr. after oral dosing, because substantial amounts of unabsorbed antibiotic were then still present. Faecal elimination of griseofulvin, as determined by the new assay, is much greater than reported earlier.

OVER the past 2 years much has been learned of the usefulness of griseofulvin as an antibiotic for treating dermatophyte infections in man and domestic animals; nevertheless, our knowledge of the degree and mechanism of its absorption from the alimentary canal remains meagre. Bedford and others (1960) conducted studies on cats and concluded that absorption of griseofulvin from the duodenum was a self-limiting phenomenon. They reported also that only 5·4 per cent of a dose administered orally to rats was detectable in the tissues at any time and that only 16 per cent was eliminated in the faeces during the 24 hr. after administration.

Having allowed for some of the absorbed griseofulvin having been destroyed by the liver, the amount calculated as remaining in the faeces was still unexpectedly low, and we therefore viewed with suspicion the ether extraction procedure used in determining faecal griseofulvin and considered it worthwhile to investigate the use of other solvents. In addition we attempted to correlate the disappearance of the antibiotic from the gut with its appearance in the blood stream and to assess the degree of absorption of single doses.

MATERIALS AND METHODS

Animals

Male albino rats of the WAG strain (140–160 g.) were used in all experiments. Animals in groups of six were given single oral doses of 10 mg. griseofulvin presented as a 1 per cent aqueous suspension in 0·5 per cent Tween 80.

Collection of Faeces

The groups of six rats were housed in cages, with grid floors, previously washed with hot water and then with acetone to remove fluorescent contaminants. The faeces, which dropped through the grid on to a washed tray, were collected at various times after dosing.

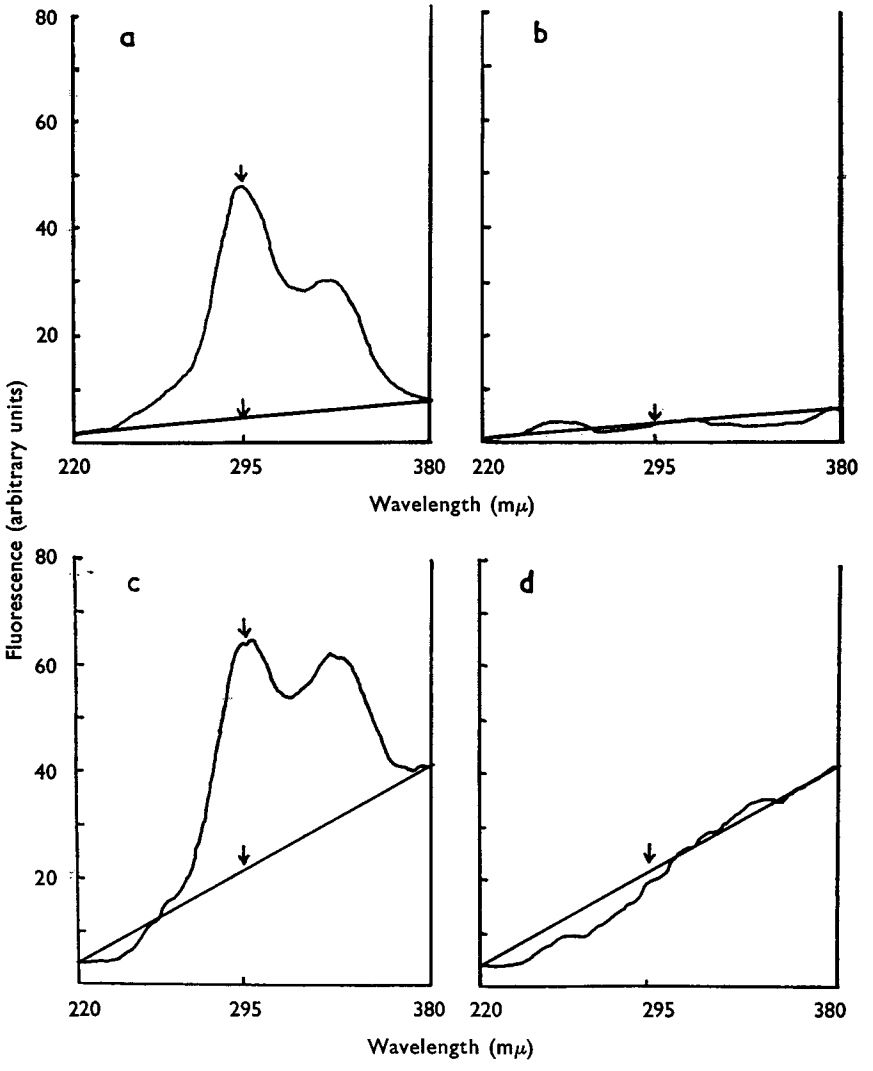

Fig. 1. (a) Scan of 1 per cent ethanol containing 0·5 μg. griseofulvin/ml.
(b) Scan of 1 per cent ethanol.
(c) Scan of small intestine extract dissolved in 1 per cent ethanol containing 0·5 μg. griseofulvin/ml.
(d) Scan of small intestine extract dissolved in 1 per cent ethanol.

(The different readings given in Fig. 1a and Fig. 2a by standard griseofulvin solution (0·5 μg./ml.) reflect changes in solution temperature or the sensitivity of the instrument.)

Estimation of Griseofulvin in Various Sections of the Alimentary Canal

Rats were killed and their entire alimentary tracts were removed. These were cut into four parts; oesophagus and stomach, small intestine, caecum, colon and rectum. The corresponding sections from all six rats in each group were bulked, finely divided with scissors and extracted thrice with acetone, once with 100 ml. and twice with 50 ml. The extracts were filtered (Whatman No. 4), and the filtrates were bulked and diluted with more acetone. The volume used was such that the solution obtained by evaporating 1 ml. of the final acetone solution and dissolving the residue in a suitable volume of 1 per cent ethanol gave a reasonable deflection on the most sensitive scale of a Farrand Spectrophotofluorometer.

This 1 per cent ethanolic solution was scanned through the activating range 380 to 220 mμ, with the analysing wavelength set at 450 mμ (uncorrected values). The intensity of the fluorescence derived from griseofulvin was calculated by joining with a straight line the scan readings at 380 and 220 mμ and subtracting the ordinate value of this straight line at 295 mμ from the total fluorescence at this wavelength. The fluorescence intensity so calculated was compared with that obtained similarly with a standard solution of griseofulvin.

The validity of this method of assessing the intensity of irrelevant fluorescence was verified by extracting tissues from control rats, scanning the extracts between 380 and 220 mμ and comparing the true blank value with that calculated by the straight line method. The two values obtained differed by not more than 2 arbitrary units, equivalent to a difference of approximately 4 per cent.

Typical scans for aqueous 1 per cent ethanol, 1 per cent ethanol containing 0·5 μg. griseofulvin/ml. and extract of small intestine with and without added griseofulvin are shown in Fig. 1.

The percentage recoveries of griseofulvin added in amounts of 10 or 60 mg. to various sections of alimentary tract were 94 $\pm$ 2·3 (four experiments) and 95 $\pm$ 4·0 (four experiments), respectively; the recoveries from the various sections were almost identical.

Estimations of Griseofulvin in Faeces

Distilled water sufficient to make a paste was added to the bulked faeces of each group, and the faecal paste was extracted once with 200 ml. acetone and thrice with 100 ml. The acetone extracts were filtered (Whatman No. 4) and bulked, and the volume was adjusted with acetone so that a reading was obtained on the most sensitive scale of the spectrophotofluorometer when 1 ml. of the final acetone solution was evaporated to dryness and the residue dissolved in 1 per cent ethanol. The concentration of griseofulvin in this solution was calculated by comparing the difference in fluorescence activities at activating wavelengths 380 and 295 mμ (analysing wavelength 450 mμ) with the activity of a standard griseofulvin solution calculated by the method previously described for the gut. This method of calculating irrelevant fluorescence derives from

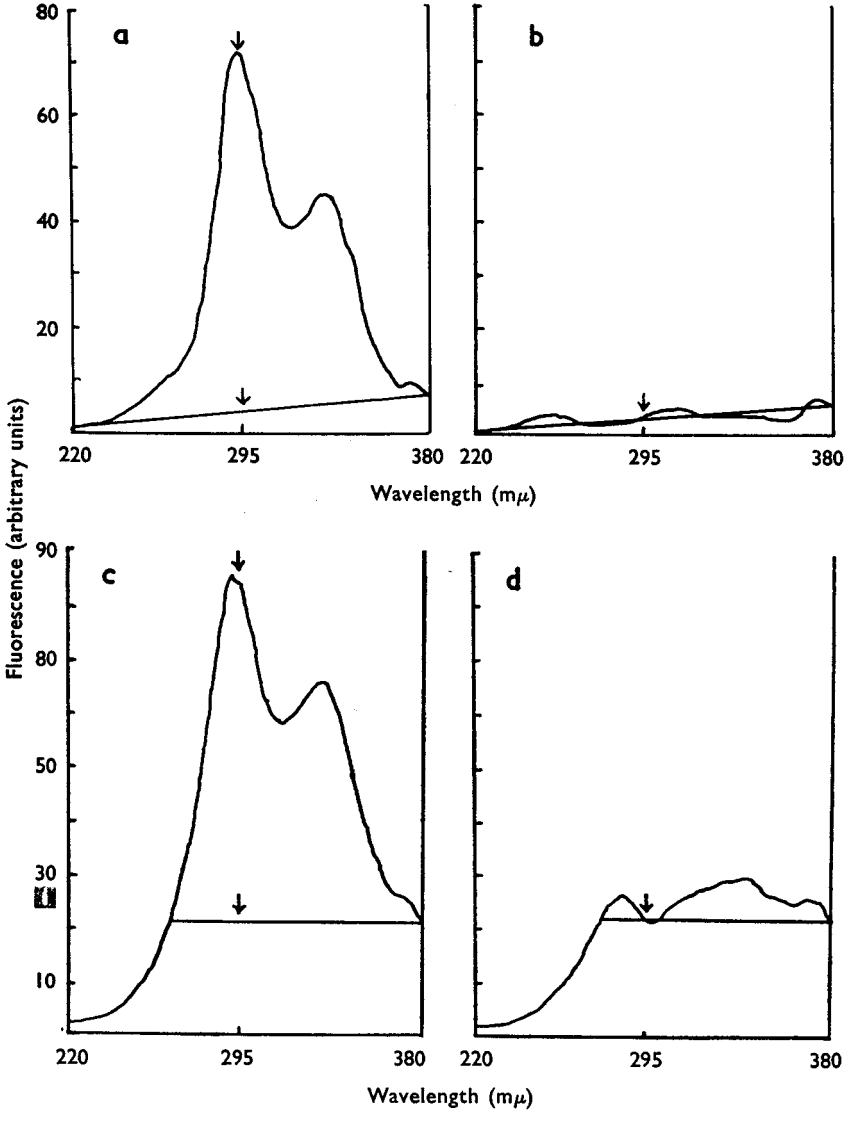

Fig. 2. (a) Scan of 1 per cent ethanol containing 0·5 μg. griseofulvin/ml.
(b) Scan of 1 per cent ethanol.
(c) Scan of faeces extract dissolved in 1 per cent ethanol containing 0·5 μg. griseofulvin/ml.
(d) Scan of faeces extract in 1 per cent ethanol.

(The different readings given in Fig. 1a and Fig. 2a by standard griseofulvin solution (0·5 μg./ml.) reflect changes in solution temperature or the sensitivity of the instrument.)

the fact that the fluorescence activities at 295 and 380 mμ were identical when faeces from controls were examined (Fig. 2).

The recovery of griseofulvin added in amounts of 30 or 60 mg. to the bulked faeces from six rats was 96 per cent.

Estimation of Griseofulvin in Blood

Blood samples were obtained from anaesthetised rats by direct cardiac puncture: heparin was added to prevent clotting (50 I.U. in 0·1 ml./3 ml. blood). The samples were assayed by the method of Bedford, Child and Tomich (1959).

Results

Alimentary Distribution and Faecal Elimination of a Single Oral Dose

The griseofulvin contents of different sections of the alimentary tract and of the faeces at various times after a single oral dose of 10 mg. are given in Table I.

The total recovery from the alimentary tract decreased with time. It will be seen that some griseofulvin spilled into the small intestine during

TABLE I

GRISEOFULVIN PRESENT IN FAECES, BLOOD AND SECTIONS OF THE ALIMENTARY TRACT OF THE RATS AT VARIOUS TIMES AFTER A SINGLE ORAL DOSE (10 mg./rat)

	Percentage of dose recovered—hr. after administration							
	0	2	4	6	8	12	18	24
Stomach	92·5	40·2	23·4	18·8	4·9	0·4	0	0
Small intestine ..	7·4	56·0	52·1	41·5	30·5	17·0	17·7	10·8
Caecum	0	0	13·2	17·0	28·8	15·0	6·2	3·6
Colon and rectum	0	0	2·4	4·7	13·1	10·0	6·8	3·1
Faeces	—	—	—	0	2·7	26·6	34·3	37·5
Total	99·9	96·2	91·1	82·0	80·0	69·0	65·0	55·0
Blood level μg/ml.	—	1·51	2·27	1·09	0·81	0·02	—	—

oral administration. Four hr. after administration 91 per cent of the dose was recovered, this figure falling to 80 per cent at 8 hr. and 65 per cent at 18 hr. Twenty-four hr. after dosing a total of 55 per cent was recovered, of which 17·5 per cent was still in the alimentary tract and 37·5 per cent in the faeces. Faeces were not collected from animals killed 2 or 4 hr. after dosing.

Blood Levels after a Single Oral Dose of 10 *mg.*

Blood levels of other rats given single oral doses of 10 mg. are given also in Table I. The blood level reached a peak at 4 hr. and no griseofulvin could be detected 12 hr. after dosing.

Discussion

Although experiments of Bedford and others (1960) on rats indicated that only a small percentage of a single oral dose of griseofulvin was

absorbed, nevertheless only 16 per cent could be recovered from the faeces in the first 24 hr. after dosing.

Because these results failed to account for the bulk of the griseofulvin, it was decided to investigate more fully its fate in the gut.

The results of the latest experiments, in which faeces and tissues were extracted with acetone, indicate that the amount of a single oral dose appearing in the faeces is much larger than had been suggested. In the first 24 hr. after a dose of 10 mg./rat the percentage of griseofulvin recovered in the faeces was 38. In similar experiments with 5 and 20 mg./rat the percentage recoveries were 30 and 64, respectively.

Immediately after oral administration most of the griseofulvin was present in the stomach, but after 2 hr. 56 per cent had passed through into the small intestine. At this time 96 per cent of the administered dose could be recovered from the alimentary canal, the concentration in the blood being 1·5 μg./ml.: at 4 hr. the corresponding figures were 91 per cent and 2·3 μg./ml. Thus up to 4 per cent had been absorbed in 2 hr. and 9 per cent in 4 hr. These values agree with the results of Bedford and others (1960), who recovered 2·6 and 5·4 per cent of the administered dose (100 mg./kg.) from the tissues of rats 2 and 4 hr., respectively, after dosing.

The amount of antibiotic recoverable from the alimentary canal continued to decrease from 4 hr. onwards at the same time as the blood level was falling. The decline in blood level cannot be attributed to the lack of alimentary griseofulvin, because substantial amounts were present throughout the entire length of the gut, 75 per cent being in the stomach and small intestine after 4 hr. Bedford and others (1960) have suggested that absorption of griseofulvin from the duodenum of the cat involves a self-limiting mechanism, and the results presented here indicate that something similar may occur in the rat.

The observation that griseofulvin continues to disappear from the alimentary canal simultaneously with the fall in blood level suggests that some destruction of griseofulvin may occur within the gut. Work is at present in progress to investigate this possibility.

References

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