COMMON DRUGS THAT MAY INVALIDATE SPECTROPHOTOFLUOROMETRIC ASSAYS OF BLOOD GRISEOFULVIN

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Studies have been made of the potential effects of aspirin, salicylic acid, phenacetin, paracetamol, codeine, caffeine, theophylline and quinine on the spectrophotofluorometric assay of griseofulvin in blood. Aspirin, salicylic acid or quinine are likely to give falsely high values. Detection of this bias by inspecting the fluorescence spectra of the extracted griseofulvin is not always possible.

Blood griseofulvin levels are easily determined by the spectrophotofluorometric method of Bedford, Child and Tomich (1959). Because the antibiotic's only known metabolite, 6-desmethylgriseofulvin, does not fluoresce, and because the extraction procedure does not remove any fluorescent substances that may be normally present in the blood, the assay has been regarded as specific. Recently, however, when estimating the blood griseofulvin levels of human volunteers, we found that one subject had unexpectedly high values; examination of her blood extract showed that the fluorescence spectrum of the griseofulvin contained in the extract was grossly distorted. On being questioned, the subject stated that she had taken a large dose (2·4 g.) of aspirin to overcome toothache.

We decided to study the effects on the griseofulvin assay of smaller doses of aspirin, and also of other drugs that might be encountered.

Experimental and Results

Griseofulvin in 1 ml. whole blood is assayed by extracting the antibiotic into ether, evaporating the extract to dryness and dissolving the residue in 10 ml. of 1 per cent aqueous ethanol. The fluorescence of this alcoholic solution is then compared with that of a standard griseofulvin solution, both solutions being activated at 295 mμ and analysed at 450 mμ (Bedford, Child and Tomich, 1959).

In Fig. 1A are shown the activating spectra obtained on a Farrand spectrophotofluorometer for 1 per cent aqueous ethanol and for griseofulvin (0·3 μg./ml.), aspirin (10 μg./ml.) and salicylic acid (0·05 μg./ml.) in this solvent. The analysing wavelength was 450 mμ, which is maximal for griseofulvin but not for the two salicylates. The activating spectrum of griseofulvin contains two peaks, one at 295 mμ and the other at 335 mμ (uncorrected values); that of aspirin or salicylic acid has one only at 300 mμ.

The activating spectra shown in Fig. 1B were obtained on extracts of blood from a volunteer who had ingested 1 g. griseofulvin 4 hr. before venipuncture. The residues from the ether extracts were dissolved in 1 per cent ethanol, 1 per cent ethanol containing 0·05 μg./ml. salicylic acid or 1 per cent ethanol containing 0·10 μg./ml. salicylic acid. It can be

seen that the intensity of fluorescence at the activating wavelength of 295 mμ was increased from 25 arbitrary units (Fig. 1B——) to 43 (Fig. 1B- - -) and that the characteristic activating spectrum of griseofulvin was undistorted. A similar result was obtained when the residue from the ether extract was dissolved in 1 per cent ethanol containing 10 μg./ml. aspirin. However, the spectrum was entirely unlike that of griseofulvin when a sufficiently high concentration of aspirin or salicylic acid was present in the aqueous ethanol (Fig. 1B · · · · ·).

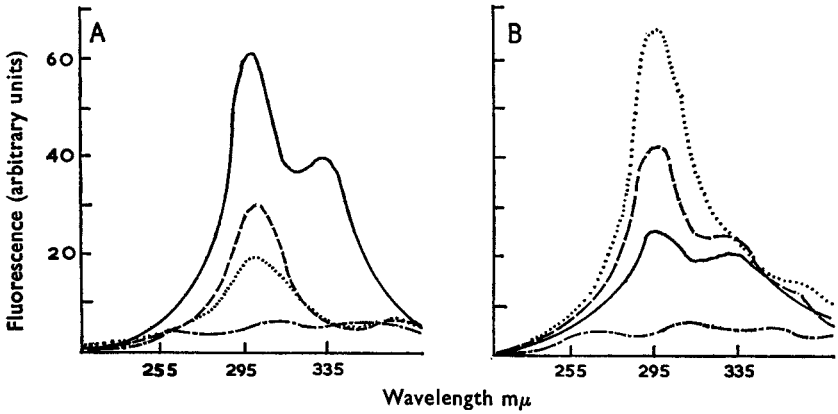

FIG. 1A. Scans of:

——— 1 per cent ethanol containing 0·3 μg. griseofulvin/ml.
. . . . . 1 per cent ethanol containing 10·0 μg. aspirin/ml.
- - - - 1 per cent ethanol containing 0·05 μg. salicylic acid/ml.
—.—. 1 per cent ethanol.

All are scans of the activation spectrum with the analysing wavelength fixed at 450 mμ

FIG. 1B. Scans of:

——— griseofulvin extracted from human blood and dissolved in 1 per cent ethanol.
- - - - griseofulvin extracted from human blood and dissolved in 1 per cent ethanol containing 0·05 μg. salicylic acid/ml.
. . . . . griseofulvin extracted from human blood and dissolved in 1 per cent ethanol containing 0·10 μg. salicylic acid/ml.
—.—. Extract of control human blood dissolved in 1 per cent ethanol.

All are scans of the activation spectrum with the analysing wavelength fixed at 450 mμ

In the experiments recorded above, salicylic acid was added to the 1 per cent ethanol used as the final solvent, but similar results were obtained when sodium salicylate was added at a concentration of 25 μg./ml. to whole blood containing griseofulvin.

Because aspirin and salicylic acid can invalidate blood griseofulvin assays, we studied the effects of other drugs that might be met in similar circumstances.

In Table I are given the fluorescence intensity values of 1 per cent aqueous ethanol solutions of griseofulvin, aspirin, salicylic acid, phenacetin, paracetamol, codeine, caffeine, theophylline and quinine; they were measured at both their own and at griseofulvin's fluorescence maxima.

Concentrations were such that readings were obtained on the most sensitive scale of the micro-ammeter. If it be assumed that a micro-ammeter reading of 10 or more units would bias the griseofulvin assay (micro-ammeter reading on normal blood extract ≡ 5 or 6 units), then it is possible to calculate for each of the above-mentioned drugs the

TABLE I

FLUORESCENCE CHARACTERISTICS OF SOME PHARMACOLOGICAL AGENTS

Compound	Concentration μg./ml.*	Fluorescence maxima mμ†		Fluorescence intensity* at maxima	Fluorescence intensity‡ at griseofulvin's maxima
		Activating wavelength	Analysing wavelength		
Griseofulvin	0·3	295	450	62	62
1 per cent aqueous ethanol	—	—	—	—	5
Aspirin	10	300	405	32	20
Salicylic acid	0·05	300	405	65	35
Phenacetin	10	385	425	25	9
Paracetamol	50	330	400	32	18
Codeine (as phosphate)	2·5	260	400	28	6
Caffeine	10	325	395	26	11
Theophylline	10	315	435	20	19
Quinine (as sulphate)	0·004	340	380	36	11

* In 1 per cent ethanol.
† Uncorrected values.
‡ Arbitrary units on the most sensitive scale of the microammeter.

TABLE II

MAXIMUM CONCENTRATIONS OF SOME DRUGS LIKELY TO BE FOUND IN HUMAN BLOOD

Drug	Normal dose range (g.)	Maximum theoretical blood concentration—μg./ml.*	Maximum blood concentration not interfering with griseofulvin assay—μg./ml.†
Aspirin	0·3 –1·0	60–200 (≡50–160 salicylic acid)	60 0·6
Phenacetin	0·3 –0·6	60–120	120
Paracetamol	0·3 –0·6	60–120	300
Codeine	0·01–0·06	2– 12	60
Caffeine	0·3 –0·6	60–120	120
Theophylline	0·06–0·2	12– 40	60
Quinine	0·3 –0·6	60–120	0·05
Tea or Coffee (per strong cup)	about 0·1 (as caffeine)	20 (as caffeine)	120

* Assuming rapid and complete absorption into a blood volume of 5 litres.
† Assuming 100 per cent recovery of drug from blood by the griseofulvin extraction procedure.

maximum blood concentration that would not falsify the results. These values, and the maximum blood concentrations likely to be encountered in practice, are given in Table II. The latter values have been calculated by assuming immediate and complete absorption into the blood stream of the maximum recommended dose.

DISCUSSION

Of the compounds tested, only aspirin, salicylic acid and quinine are likely to interfere in blood griseofulvin estimations. Lolli and Smith (1946) have shown that fasting subjects given 0·6 or 1·6 g. aspirin have maximum blood salicylate levels of 38 and 73 μg./ml. respectively. The ether extraction procedure in the griseofulvin assay of Bedford and others (1959) will remove 3 per cent of any salicylate present in the blood;

hence blood salicylate levels higher than 15 μg./ml. will falsify the griseofulvin estimations.

A given oral dose of quinine produces widely different blood levels in human subjects. One hour after a single dose of 0·5 g. a maximum concentration of about 10 μg./ml. is reached; this decreases to about 1·5 μg./ml. after 24 hr. (Sollman, 1957). Because normal doses of proprietary preparations containing quinine have about 30 mg. of the alkaloid, blood levels of 0·5 μg./ml. are theoretically possible. At this blood level 100 per cent of the quinine can be recovered in the griseofulvin assay of Bedford and others (1959), and it thus seems likely that quinine also could interfere in this assay.

The evidence indicating that salicylic acid can invalidate blood griseofulvin assays should be borne in mind when other spectrophotofluorometric assays are being conducted. When characterisation of the minute quantities of drug being assayed depends entirely on the appearance of its fluorescence spectra, then it is not enough just to ensure that metabolites and substances closely related to the drug do not fluoresce; compounds that, though not distorting the spectra, raise the peaks being measured must be taken into account.

References

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