Fifty-four derivatives of these drugs have been prepared in a systematic manner, of which 52 have not been reported to date in the literature.

A series of photomicrographs have been included in Figs. 1–3, together with a summary of the conditions under which they were formed (Table II).

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Particle Size of Commercial Griseofulvin with Reference to Official Standards

By B. A. MATTHEWS and C. T. RHODES

Samples of commercial griseofulvin have been obtained from various manufacturers and their particle size distribution has been determined by means of the Coulter counter. These results have been compared with the requirements of the monographs in the B.P., U.S.P., and the proposed specification of the "International Pharmacopoeia" and, in the light of this comparison, suggestions for improvement of these specifications have been made.

GRISEOFULVIN (7-chloro-4,6-dimethoxycoumaran - 3 - one - 2 - spiro - 1′ - [2′ - methoxy-6′-methylcyclohex-2′-en-4′-one]) has gained widespread acceptance in the oral treatment of fungal infections in man (1). It is the subject of monographs in a number of pharmacopeias (2–6). Early work on serum and tissue levels of the drug in rats, by Bedford *et al.* (7), and in humans, by McNall (8), suggested that the drug is poorly absorbed from the gastrointestinal tract. Further confirmation of this was published by Sharpe and Tomich (9), who were unable to determine the lethal dose of orally administered griseofulvin in rats and mice and by Gonzales-Ochoa *et al.* (10), who attempted to treat dermatophytoses in man.

All drugs, whether readily or sparingly soluble, dissolve more rapidly when finely divided, and Nelson (11, 12) showed that for theophylline salts and tetracycline, the rate of appearance of the drug in the blood stream following oral administration was determined by the solution rate. Griseofulvin is almost insoluble in water (13) and several attempts have been made to determine the effect of particle size on blood levels in animals (14) and man (15–17). Kraml *et al.* (17) found that a 0.5-Gm. dose of micronized griseofulvin produced serum levels indistinguishable from those produced by a 1.0-Gm. dose of nonmicronized griseofulvin. The monograph in the "British Pharmacopoeia" 1963 (2) recognizes two grades of griseofulvin called, respectively, "coarse particle" and "fine particle," whereas the "United States Pharmacopeia" (4) specifies one called "microsize," which corresponds approximately to the "fine particle" of the "British Pharmacopoeia." In each case some particle size requirements are given. The Food and Drug Administration monograph on microsize griseofulvin specifies additional particle size requirements based on specific surface area measurements by an air permeability technique. The limits are 1.3 to 1.6 sq. M./Gm. The proposed monograph for griseofulvin in the Volume of Specifications of the second edition of the "International Pharmacopoeia," to be published later this year, has a particle size specification based on a sieve technique. A limit of 5% by weight is allowed to be retained on a 300-mesh sieve (aperture size 53 μ).

The authors have obtained samples of commercial griseofulvin from several sources and by means of the Coulter counter (18, 19) have examined these to determine their particle size distribution. They have also utilized microscopy and specific surface area measurements to check results and have compared these with the particle size requirements of the pharmacopeias.

EXPERIMENTAL

Apparatus—A Coulter counter model B[1] with 50-μ and 140-μ orifices was used to determine the particle size. The electrolyte was 1% sodium

Received December 27, 1966, from the Physical Pharmacy Department, School of Pharmacy, Portsmouth College of Technology, Portsmouth, Hampshire, England.
Accepted for publication April 19, 1967.
The authors acknowledge the generosity of Glaxo Laboratories Ltd., Imperial Chemical Industries Ltd., and Ayerst Laboratories Division of American Home Products Corp. for supplying samples of griseofulvin.

[1] Marketed by Coulter Electronics Ltd., Dunstable, Bedfordshire, England.

TABLE I—RESULTS OF A SURVEY OF THE MANUFACTURERS OF GRISEOFULVIN IN THE UNITED KINGDOM, EUROPE, AND THE U. S.

Mfr.	Name	Griseofulvin, Coarse	Griseofulvin, Fine
A	Glaxo Laboratories Ltd., Middlesex, England	Mfd.	Mfd.
B	Imperial Chemicals Limited, Pharmaceuticals Division, Cheshire, England	Mfd.	Mfd.
C	Schering Corp., Bloomfield, N. J.	Obtained from	mfr. *A*
D	McNeil Laboratories, Inc., Fort Washington, Pa.	Obtained from	mfr. *A*
E	Ayerst Laboratories Division of American Home Products Corp., New York, N. Y.	Obtained from mfr. *B*	Mfd. from coarse material obtained from mfr. *B*
F	Rhein-Pharma Arzneimittel G.m.b.H., Heidelberg, Germany	Obtained from	mfr. *B*
G	Laboratories Clin-Comar, Paris, France	Obtained from	mfr. *A*
H	Laboratori Glaxo S.p.A., Verona, Italy	Obtained from	mfr. *A*

chloride and polysorbate 80[2] was used as a dispersing agent, in conjunction with ultrasonic vibration.[3] The 50-μ orifice was calibrated with 1.305-μ diameter polystyrene-latex[4] and also by 4.6-μ diameter puff-ball spores. Good agreement was achieved between the respective calibration constants. The 140-μ orifice tube, which was used for the coarse material, was calibrated with 13.6-μ paper mulberry pollen. The electrolyte was filtered through a 0.45-μ Millipore[5] filter, and background counts at all settings of the Coulter were always well below the recommended limits.

Materials—Samples of fine and coarse griseofulvin were requested from two manufacturers in the United Kingdom, three in the United States, and one in France, West Germany, and Italy. The results of the inquiries are given in Table I. The authors subsequently obtained several samples of different batches of griseofulvin from manufacturers *A*, *B*, and *E* and submitted these to analyses on the Coulter counter.

Method—For each measurement a concentrated suspension was prepared in electrolyte, which had been previously saturated with griseofulvin, and this was subjected to ultrasonic dispersion. Dilutions were made in saturated electrolyte to a suitable working strength and counts were taken at the lowest settings at the beginning and end of the determination to ensure that flocculation did not occur.

Mounts were prepared for microscopy by using ultrasonic dispersion to prepare a concentrated suspension of discrete particles and this was mounted in glycerin containing polysorbate 80. Photographs were taken as a check on particle size. Specific surface area measurements were made on the Fisher sub-sieve sizer[6] which is based on the work of Gooden and Smith (20).

The data from Kek Ltd. was supplied in the form of tables of mean particle diameter against porosity. Since the mean particle diameters reached a minimum at a porosity of 0.425 in each case, it was considered justifiable to make comparisons of specific surface at this value using the formula given by Pendleton (21).

[2] Marketed as Tween 80 by Honeywill-Atlas Ltd., London, England.
[3] M.S.E. Ultrasonic Disintegrator, model 60w, marketed by Measuring and Scientific Equipment Ltd., London, England.
[4] Kindly supplied by Mr. B. J. Lippie, Dow Chemical Co., Midland, Mich.
[5] Marketed by Millipore Ltd., Middlesex, England.
[6] The authors are grateful to Mr. P. D. Hines, Kek Ltd., Manchester, England, for specific surface area measurements.

RESULTS AND DISCUSSION

Fine Particle Griseofulvin—Three determinations were performed on separate samples of batch 1 of fine particle griseofulvin from manufacturer *B*, to determine the reproducibility of repeated measurements. The results of this experiment are given in Table II. Since the count was still increasing at the lowest settings of the instrument, the 100% level was determined by extrapolating the curve of log $\Sigma(\Delta n)\ \bar{v}$ against diameter d for the last six points as recommended in the Coulter instruction manual (22).

These results show good agreement especially at the middle and fine end of the particle size distribution. There is more variation at the coarse end, where only a few particles are counted at each size, and consequently sampling errors tend to be greater. Counts were then performed on three separate batches from manufacturer *A*, three from manufacturer *B*, and one from manufacturer *E*. These are shown in Table III. Extrapolation was performed on the data from all the batches from manufacturer *B* and for that from *E* for the reasons given above. The size distribution of the material obtained from

TABLE II—REPRODUCIBILITY OF REPLICATE DETERMINATIONS OF PARTICLE SIZE DISTRIBUTION ON BATCH 1 OF FINE PARTICLE GRISEOFULVIN FROM MANUFACTURER *B*

Equivalent Spherical Diam., μ	Cumulative Wt. % above Stated Size 1	2	3
16.96	2.25	1.71	2.98
13.46	5.64	3.43	5.96
10.69	9.03	6.43	7.45
8.48	10.16	9.00	10.43
6.73	13.83	11.03	13.60
5.34	16.44	14.62	16.58
4.24	20.50	18.82	20.17
3.37	27.50	27.66	27.18
2.67	42.19	45.03	42.01
2.12	65.71	67.00	64.19
1.68	85.15	82.57	82.14
1.34	92.83	88.17	89.91
1.06	97.15	95.00	95.17
0	100.0	100.0	100.0

TABLE III—PARTICLE SIZE DISTRIBUTION OF DIFFERENT BATCHES OF FINE PARTICLE GRISEOFULVIN FROM MANUFACTURERS *A*, *B*, AND *E*

Equivalent Spherical Diam., μ	Cumulative Wt. % Above Stated Size						
	Mfr. *A*			Mfr. *B*			Mfr. *E*
	1	2	3	1	2	3	1
16.96	0.72	0.75	0.57	2.25	0	2.21	2.46
13.46	2.15	1.18	1.72	5.64	2.12	6.62	9.43
10.69	3.58	3.20	3.29	9.03	4.94	7.73	14.97
8.48	5.99	5.75	5.52	10.16	9.70	11.86	25.85
6.73	10.10	9.94	9.06	13.83	13.24	14.75	35.49
5.34	18.17	19.48	16.90	16.44	17.56	19.79	46.70
4.24	36.10	41.76	37.54	20.50	23.51	28.45	59.25
3.37	61.46	66.62	64.98	27.50	37.83	43.80	70.57
2.67	82.17	84.67	85.56	42.19	59.05	46.65	81.20
2.12	93.09	94.27	95.24	65.71	78.93	71.40	89.21
1.68	98.43	98.35	99.01	85.15	91.02	87.29	95.58
1.34	99.38	99.52	99.74	92.83	94.85	92.70	97.89
1.06	100.0	100.0	100.0	97.15	97.61	96.82	99.27
0	...	...	...	100.0	100.0	100.0	100.0

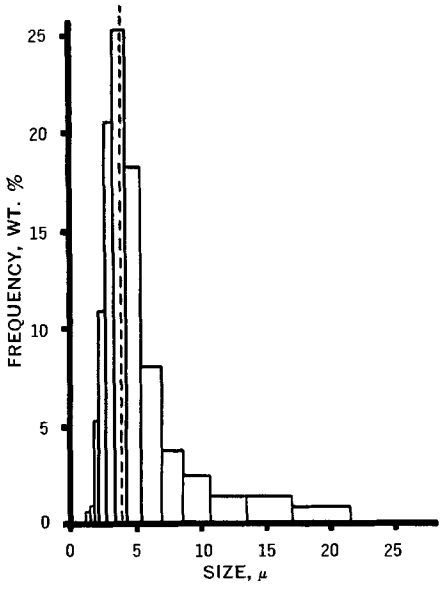

Fig. 1—Histogram plot of particle size distribution of batch 1 of fine particle griseofulvin from manufacturer A.

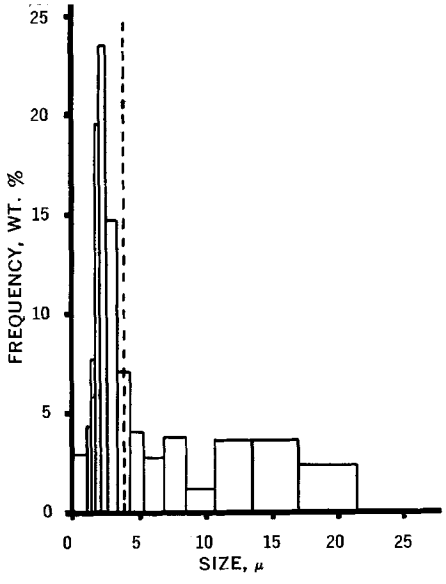

Fig. 2—Histogram plot of particle size distribution of batch 1 of fine particle griseofulvin from manufacturer B.

manufacturer *A* appeared to cut off at around 1 μ, which corresponded to the lowest setting on the instrument, and extrapolation was not considered necessary in this case. The size distribution of the three batches from manufacturers *A* and *B* appears to fall into a definite and distinctive pattern with little between-batch variation, suggesting that good control is achieved by each manufacturer.

Histograms of representative batches of material from manufacturers *A* and *B* and of the sample from manufacturer *E* are given in Figs. 1–3. In addition, plots of cumulative weight per cent above stated size against size using the mean of the values for the three batches from manufacturers *A* and *B* are given in Fig. 4. The "British Pharmacopoeia" monograph on griseofulvin (2) states that for the fine particle material "the particles are mostly less than 4 μ in diameter with only occasional particles exceeding 5 μ." The "United States Pharmacopeia" (4) states that "particles of the order of 4 μ pre-

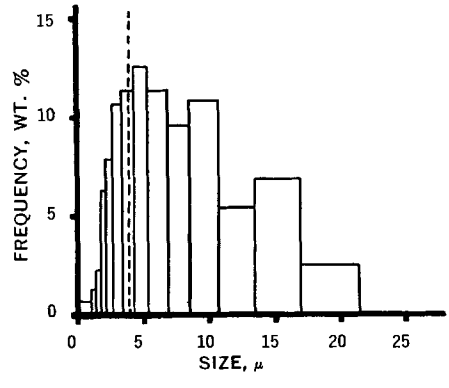

Fig. 3—Histogram plot of particle size distribution of batch 1 of fine particle griseofulvin from manufacturer E.

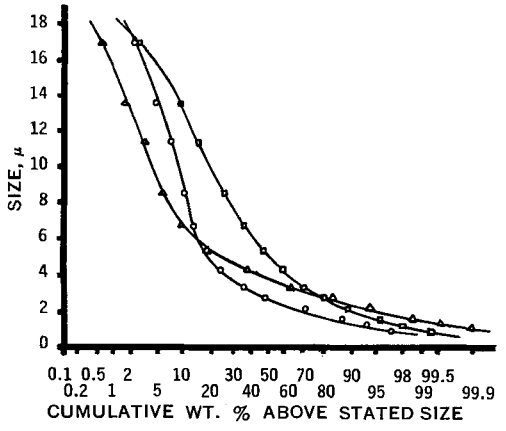

Fig. 4—Plot of cumulative weight per cent above stated size against size for fine particle griseofulvin. Key: △, mean of three batches from manufacturer A; ○, mean of three batches from manufacturer B; □, batch from manufacturer E.

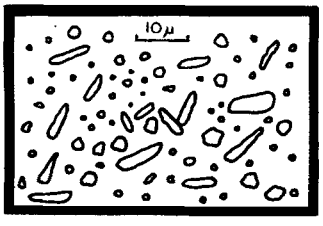

Fig. 5—Diagram of a typical field of fine particle griseofulvin taken from a photomicrograph.

TABLE IV—SPECIFIC SURFACE AREA OF FINE PARTICLE GRISEOFULVIN

	Mfr. *B* Sample 1	Mfr. *A* Sample 1	Mfr. *E*
Specific surface area, sq. M./Gm.	1.54	1.32	1.23
Peak of size distribution histogram	2.5 μ	3.5 μ	5 μ

TABLE V—PARTICLE SIZE DISTRIBUTION OF COARSE PARTICLE GRISEOFULVIN

Equivalent Spherical Diam., μ	Cumulative Wt. % Above Stated Size Mfr. *A*	Mfr. *B*
67.9	0	4.43
53.8	0	8.86
42.8	7.26	14.40
33.9	12.70	19.96
26.9	16.33	28.82
21.4	23.13	39.34
16.9	31.98	51.12
13.5	42.81	63.84
10.7	52.10	74.56
8.5	62.92	82.08
6.7	71.12	87.55
5.3	80.00	91.51
4.2	87.39	94.70
3.4	93.15	97.03
2.7	95.60	98.07
2.1	98.22	99.65
0	100.0	100.0

dominate." It is not entirely clear from these statements whether the specifications refer to a number distribution or a weight distribution. For powdered drugs the latter is obviously more important since a very few particles at the upper end of the distribution can affect the weight per cent distribution considerably.

When the results in Figs. 1–3 are compared with the above requirements, it is seen that the peak of the size distribution curve is at about 2.5 μ for the material from manufacturer *B*, 3.5 μ for that from *A*, and 5 μ with that from *E*. It was found that almost 60% by weight of the sample from this latter manufacturer was above the 4 μ limit. The B.P. requirement that only an occasional particle should be above 5 μ may be true on a number basis, but the results show that the samples from manufacturers *A* and *B* showed about 20% by weight above 5 μ and that from *E* almost 50%.

Shape Factor—Since the diameter obtained with the Coulter counter is a mean spherical diameter and the authors knew that many of the particles in fine particle griseofulvin were acicular, photographs of several samples of this material were taken. A drawing of a typical field taken from a photograph is shown in Fig. 5. From this it may be observed that the average length/breadth ratio of the needles is about 3. No mention of shape is made in the size requirements of the pharmacopeias and one dimension of the particle may be much larger than the Coulter diameter. For example a sphere of 4 μ diameter would have approximately the same volume as a cylinder of diameter 2.5 μ and length 7.5 μ. Allowing for this the results of microscopic measurements were in good agreement with the Coulter data. The length of the acicular particles is small compared with the dimensions of the sensitive volume of the orifice, and we have been informed (23) that despite the shape factor the pulse height will be a true measure of the particle volume.

Specific Surface Area Measurements—The papers (15–17) which have related the absorption of griseofulvin to its particle size have used the parameter, specific surface area. This was therefore determined for a sample of griseofulvin from each manufacturer. The results are given in Table IV.

The shape of the histograms in Figs. 1–3 are reflected in the results of these specific surface area measurements. The lower value of the modal particle size of the material from manufacturer *B* is to some extent counterbalanced by the existence of another peak between 10 and 20 μ, and so the difference in specific surface area between samples *A* and *B* is not as would otherwise have been expected. The coarsest sample *E* had a specific surface area of 80% of that of the finest *B*. Atkinson *et al.* (15) have shown a close correlation between specific surface area of griseofulvin and absorbability, but it is not possible to say whether the differences found in the samples above would be clinically significant.

Coarse Particle Griseofulvin—Owing to the relative economy of treatment with fine particle griseofulvin, both manufacturers *A* and *B* have ceased to market the coarse particle material. The authors have, however, obtained samples from each and the results of size distribution analyses with the Coulter counter are given in Table V and Fig. 6.

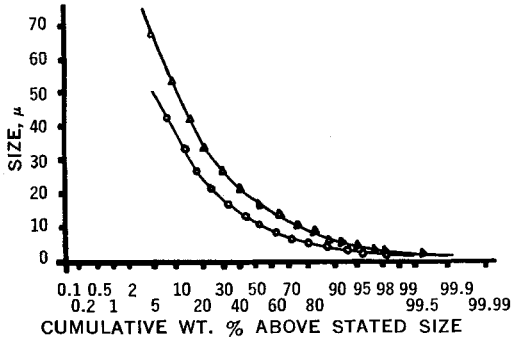

Fig. 6—Plot of cumulative weight per cent above stated size against size for coarse particle griseofulvin. Key: O, manufacturer A; △, manufacturer B.

TABLE VI—SPECIFIC SURFACE AREA OF COARSE PARTICLE GRISEOFULVIN

	Mfr. *A*	Mfr. *B*
Specific surface area, sq. M./Gm.	0.38	0.48

No specification is given in the U.S.P. for this material, but the B.P. states that the particles are "mostly about 30 μ in diameter with occasional particles up to 60 μ." The proposed monograph for the 2nd "International Pharmacopoeia" (24) does not specify whether the type of material referred to is coarse or fine. From the particle size test which allows 5% by weight to be retained by a sieve with a mesh size of 53 μ, it appears that the material is similar to coarse particle griseofulvin of the B.P.

The sample from manufacturer *A* has no particles with an equivalent spherical diameter greater than the maximum permitted value but the peak of the size distribution is around 15 μ. The sample from manufacturer *B* has a few particles coarser than the permitted maximum but its size distribution peak is also around 15 μ. The greater percentage of coarse material in this sample is reflected in the specific surface area measurements given in Table VI.

The sample from manufacturer *A* has no particles with a Coulter diameter greater than the 53-μ mesh diameter specified in the "International Pharmacopoeia," whereas that from manufacturer *B* had 8.9% above this limit. However, as mentioned earlier, the particles are often acicular and consequently such particles with an equivalent spherical diameter greater than 53 μ could pass through a sieve of this mesh size.

SUMMARY

Samples of griseofulvin available commercially from different sources exhibit considerable differences in particle size distribution. Because of the imprecise nature of the official standards in the pharmacopeias, a wide range of size distributions can be considered as meeting their requirements. The authors have shown that the Coulter counter can be used to determine the particle size distribution of this drug and because of the shape factor feel that the methods used should be specified by the pharmacopeias. Because of the relationship between blood levels and particle size, it is felt that more stringent specifications should be given. It is suggested that a modal diameter of the distribution expressed as a weight percentage with upper and lower limits controlling the amount outside them would be more effective in controlling the particle size of this material. Because of the relationship between specific surface and blood levels, it is felt that all pharmacopeias should also include some limits of this parameter.

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