Effect of Griseofulvin Upon Lipid Films at the Air-Water Interface

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The effect of griseofulvin upon monomolecular films of stearic acid and cholesterol has been determined by comparing the pressure-area curves obtained when these film-forming materials were spread on a redistilled water substrate and one consisting of a solution of griseofulvin in redistilled water.

IN CONNECTION with studies on the structural requirements and physicochemical characteristics associated with a compound's capacity for anchoring to dermal tissue constituents or for enhancing its localization in the latter, the authors sought to determine the effects of griseofulvin upon monomolecular films of two representative lipid dermal constituents (1), *i.e.*, stearic acid and cholesterol.

EXPERIMENTAL

Materials—Stearic acid was obtained in purity greater than 99%, m.p. 71.1–72.0°.[1] Chromatographically pure cholesterol (99 +%) was procured (Sigma Chemical Co.), m.p. 148.8–149.4°. Griseofulvin was recrystallized from benzene, yielding white needles, m.p. 220.5–221.4°; $[\alpha]_D^{26}$ +331.03° (c 1.015 in acetone); $\lambda_{max.}^{EtOH}$ 215 mμ (ε 23,107), 235 (22,754), 292 (23,636), 330 (5,644); $\lambda_{max.}^{CHCl_3}$ 5.85 μ (C=O), 6.02 (COC=C). Its properties were consistent with those reported in the literature (2, 3).

Anal.[2]—Calcd. for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86; Cl, 10.05. Found: C, 57.79; H, 4.78; Cl, 10.30.

The substrate water was obtained by redistillation through a 45-cm. Vigreaux column from aqueous permanganate (4). It had a pH of 6.0, and gave a value of 0.15 p.p.m. in conductometric measurements of ionizable substances expressed as sodium chloride. Solutions of griseofulvin (20 mcg./ml.; $5.7 \times 10^{-5} M$) were prepared by boiling the substrate water to which griseofulvin had been added (5); the absorbance at 295 mμ for the solutions prepared in this manner (5) was 1.35 ± 0.02.

Instrumentation and Methods—The surface balance utilized is a refinement of the commercially available Hydrophil balance (Central Scientific Co.) (see Fig. 1). The trough and all Teflon parts of the apparatus which come into contact with the substrate and monolayer were thoroughly cleaned with reagent grade benzene followed by rinsing with spectro-grade benzene and redistilled water. The equipment was assembled and used within an isolation cabinet (Kewaunee Scientific Equipment). All experiments were carried out at room temperature (substrate temperature, 24 ± 2°).

After the cleanliness of the substrate surface was checked, the compression barrier was positioned (initial area, 490–560 cm.²), and the film-forming material was applied to the surface of the substrate by means of an Agla micrometer-syringe (Burroughs Wellcome & Co.), in spectro-grade benzene (0.125–0.176 μmole/0.05–0.10 ml.). Following a period of 20 min. during which the solvent evaporated, the compression barrier was moved slowly toward the float. Usually 2-mm. decrements were employed, starting about 3 cm. before pressure was first observed; as the pressure increased rapidly with a small decrease in area, 1-mm. decrements were used. In each case, there was a 1-min. interval between the time when the barrier was stopped and that when the reading of surface pressure was made.

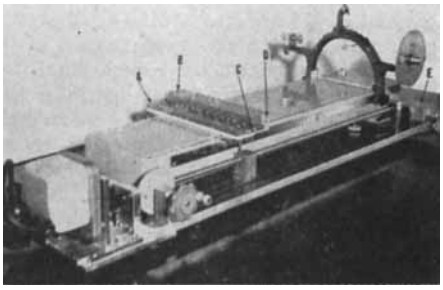

Fig. 1—The modification of the surface balance. The trough is Teflon coated, and the float and end piece systems, patterned after those of Mann and Hansen (6), are constructed of Teflon. Key: A and B, compression barrier units (solid Teflon attached to solid brass) transported at uniform rates by motor-driven operation; C, restraining arm for part B; D, scale pointer; E, switch providing automatic cessation of barrier movement.

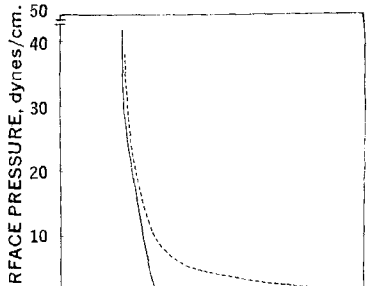

Fig. 2—Surface pressure vs. *area per molecule for stearic acid spread on: redistilled water (———); 5.7 × 10⁻⁵ M solution of griseofulvin in redistilled water (– – – –). In the latter case the corrected surface pressure is shown.*

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[1] Melting points are corrected; they were determined with a Büchi melting point apparatus.

[2] Analyses were performed by Drs. G. Weiler and F. B. Strauss, Oxford, England.

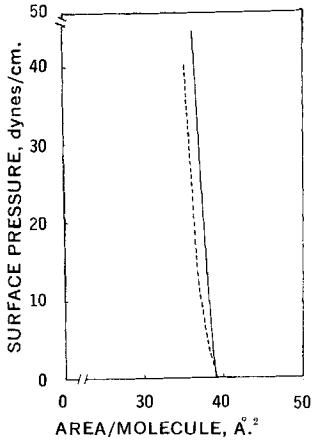

Fig. 3—Surface pressure vs. *area per molecule for cholesterol spread on: redistilled water (———); 5.7 × 10⁻⁵ M solution of griseofulvin in redistilled water (- - - -). In the latter case the corrected surface pressure is shown.*

After the film collapsed, a check was made for monolayer leakage.

RESULTS AND DISCUSSION

Each of the pressure-area isotherms (Figs. 2 and 3) was obtained by averaging the results of three independent experiments; the experimental points deviated from the curve average generally by no more than 0.2 Å.² per molecule, at a given surface pressure. It should be noted that the pressure-area curves for stearic acid and cholesterol spread on water agree well with those reported by Walker and Ries (7) and Taylor and Haydon (8). The surface pressure values for stearic acid and cholesterol, spread on the aqueous solution of griseofulvin, are corrected.[3] This permitted direct comparison with values obtained when the substrate was redistilled water.

The broken line in Fig. 2 indicates that griseofulvin was incorporated into the monolayer, and that, as the pressure increased, most of it was forced out of the film. The collapse pressure for this curve was slightly lower than that for pure stearic acid; this appears to be consistent with the observation (10) that the presence of irregularly shaped molecules in films of regularly oriented molecules renders the film less stable.

[3] The correction factor (8, 9) was determined to be 0.6 dyne/cm. using a du Nouy tensiometer and employing the ring method by procedures previously described (4).

The curve for cholesterol spread on the solution of griseofulvin (Fig. 3) is displaced slightly toward a lower area per molecule compared with that spread on redistilled water. This suggests that griseofulvin either facilitates a closer packing of the cholesterol molecules than that in the film of the pure substance, or that griseofulvin causes some of the cholesterol molecules to leave the area-determining positions in the surface; the latter may be, possibly, due to a process of interfacial dissolution (10).

The data indicate a somewhat limited but unequivocally significant affinity of griseofulvin for stearic acid. This is interesting in the light of evidence (5, 11) that the presence of lipid material in skin tissue may be associated with uptake of griseofulvin. While the authors are aware of the fact that a response to a given compound *in vitro* cannot be interpreted as evidence of the latter's corresponding involvement *in vivo*, even without conclusive causal connection, one should not underestimate the importance of leads of this nature. In the instance of the interactions observed in the cholesterol monolayer, one is tempted to interpret the isotherm also in terms of a type of affinity; it is possible to visualize that some of the cholesterol molecules shifted from their area-determining positions toward the aqueous phase because of their affinity for the griseofulvin.

Studies concerned with the affinity of a series of grisan derivatives for stearic acid and other lipid constituents have been recently completed (12, 13). They are expected to be supplemented with complementary studies.[4]

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