dissolved in aqueous acetic acid, and the product was precipitated by addition of ethanol.

Compound IV was an amorphous solid (59% yield) which did not melt up to 300°C. $^1$H-NMR ($D_2O$): δ 2.50 (m, $CH_2P$), 3.48 (m, CHCO), 4.04 (s, $OCH_3$), 4.58 (m, Ar$CH_2$), and 7.63 ppm (m, ArH). IR (KBr): 3700–2500 (NH and OH), 1680, 1740 (C=O), 1200 ($OCH_3$), and 1070 $cm^{-1}$ (P=O).

*Anal.*—Calc. for $C_{13}H_{19}N_2O_6P \cdot 3.5\ H_2O$: N, 7.12; P, 7.87. Found: N, 6.64; P, 8.33.

Compound V was an amorphous solid (55% yield) which did not melt up to 300°C. $^1$H-NMR ($D_2O$–$D_2SO_4$): δ 2.00–5.10 (m, aliphatic H) and 7.70 ppm (ArH); IR (KBr): 3700–2500 (NH and OH), 1650–1660 (C=O), and 1070 $cm^{-1}$ (P=O).

*Anal.*—Calc. for $C_{12}H_{17}N_2O_6P$: N, 8.86; P, 9.80. Found: N, 8.18; P, 10.25.

Compound VIII was an amorphous gum which softened without melting at 90°C. TLC on silica gel (methanol) showed one spot, $R_f$ 0.5.

Compound IX was an amorphous solid (50% yield) which did not melt up to 260°C. $^1$H-NMR ($D_2O$–$D_2SO_4$): δ 2.75–3.88 (m, CH and $CH_2$), 4.38–5.25 (m, Ar$CH_2$ and CH), and 7.62 $cm^{-1}$ (s, ArH). IR (KBr): 2700–3500 (OH, ArH, and CH), 1700 (C=O), 1150 (C—O), and 1050 $cm^{-1}$ (P=O).

*Anal.*—Calc. for $C_{12}H_{17}N_2O_6P \cdot H_2O$: N, 8.38; P, 9.26. Found: N, 8.40; P, 9.02.

## REFERENCES


(1) R. H. Mazur, J. M. Schlatter, and A. H. Goldcamp, *J. Am. Chem. Soc.*, **91**, 2684 (1969).

(2) R. H. Mazur, K. A. Reuter, R. A. Switek, and J. M. Schlatter, *J. Med. Chem.*, **16**, 1284 (1973).

(3) S. A. MacDonald, C. G. Wiulson, M. Chorev, F. S. Vernacchia, and M. Goodman, *J. Med. Chem.*, **23**, 413 (1980).

(4) J. W. Anderson and L. Fowden, *Chem. Biol. Interactions*, **2**, 53 (1970).

(5) P. Mastalerz, *Arch. Immunol. Exp. Ther.*, **7**, 201 (1959).

(6) (a) J. W. Huber and W. F. Gilmore, *J. Med. Chem.*, **18**, 106 (1975); (b) J. G. Allen, F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, L. J. Nisbet, and P. S. Ringrose, *Nature (London)*, **272**, 56 (1978); (c) J. R. Atherton, M. J. Hall, C. H. Hassall, R. W. Lambert, and P. S. Ringrose, *Microb. Agents Chemother.*, **15**, 677 (1979).

(7) J. R. Vaughn, Jr., *J. Am. Chem. Soc.*, **73**, 3547 (1951).

(8) W. F. Gilmore and H. A. McBride, *J. Pharm. Sci.*, **63**, 1087 (1974).

(9) P. Kafarski, B. Lejczak, P. Mastalerz, J. Szewszyk, and C. Wasielewski, *Can. J. Chem.*, **60**, 3081 (1982).

(10) P. M. Bryant, R. H. Moore, P. J. Pimlott, and G. T. Young, *J. Chem. Soc.*, **1959**, 3868.

(11) S. Asano, T. Kitahara, T. Ogawa, and M. Matsui, *Agric. Biol. Chem. Jpn.*, **37**, 1193 (1973).

(12) M. Fujino, M. Wakimasu, K. Tanaka, H. Aoki, and N. Nakajima, *Naturwissenschaften*, **60**, 351 (1973).

(13) M. Kawai, M. Chorev, J. Marin-Rose, and M. Goodman, *J. Med. Chem.*, **23**, 420 (1980).

(14) M. Soroka and P. Mastalerz, *Rocz. Chem.*, **50**, 661 (1976); *Chem. Abstr.*, **81**, 151443v.

(15) M. Hoffman and C. Wasielewski, *Rocz. Chem.*, **50**, 139 (1976); *Chem. Abstr.*, **84**, 180338g.

(16) K. D. Berlin, N. K. Roy, R. T. Claunch and D. Bude, *J. Am. Chem. Soc.*, **90**, 4494 (1968).

(17) R. Boissonnas and G. Preitner, *Helv. Chim. Acta.*, **36**, 875 (1953).


## ACKNOWLEDGMENTS


This work was performed under an exchange program between the University of Oregon and the Technical University of Wroclaw, Wroclaw, Poland, sponsored by the National Science Foundation. The authors thank the Technical University of Wroclaw for providing facilities and financial support and the University of Oregon for allowing one of us (V.N.) to participate in the program.


# Induction of Hyperpyrexia by Dihydrocurvularin, a Metabolic Product of *Penicillium gilmanii*


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Received May 24, 1983, from the *Department of Chemistry, American University, Washington, DC 20016.* Accepted for publication March 12, 1984.



**Abstract** □ The previously reported rise in rectal temperature that follows the intravenous injection of the mixture of metabolic products (extractable with ether from the Czapek Dox medium on which *Penicillium gilmanii* has grown) is due to a single compound, dihydrocurvularin. Intravenous injection of 1–10 μg of dihydrocurvularin into rabbits causes a rise of at least one degree in rectal temperature of rabbits in 2–8 h. The degree of temperature rise depends more on the individual rabbit than on the quantity of dihydrocurvularin injected. Treatment with lipopolysaccharide abolishes the ability of dihydrocurvularin to cause a rise in rectal temperature. Treatment with dihydrocurvularin, however, does not abolish the ability of lipopolysaccharide to induce a temperature response or a leukocytosis. Rabbits respond to repeated treatment with dihydrocurvularin with a rise in rectal temperature that is indistinguishable from that observed on their first injection. Treatment with dihydrocurvularin does not affect differential counts or the concentration of leukocytes or red blood cells in the circulatory system.



**Keyphrases** □ Dihydrocurvularin—intravenous injection, hyperpyrexia, rabbits, effect of lipopolysaccharide


The intravenous injection of microgram quantities of the mixture of metabolic products elaborated by *Penicillium gilmanii* grown on a Czapek Dox medium induces a temporary rise in rectal temperature and leukocytosis in rabbits (1, 2). After extraction with ether the medium no longer induced a temperature rise but afforded a pure crystalline enolic heterocyclic compound, leucogenenol (2), of which 0.002 μg/kg, on intravenous injection, induces a temporary leukocytosis in animals (1, 3), but even milligram quantities do not induce hyperpyrexia. On the other hand, intravenous injection of microgram quantities of the mixture of compounds extractable from the culture medium with ether induces a hyperpyrexia in rabbits comparable to that observed following the injection of microgram quantities of lipopolysaccharides.

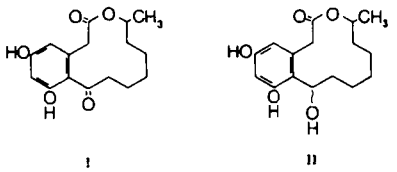
Investigations on leucogenenol established that it acted on cells in the peripheral circulation by increasing the rate at which committed cells of the bone marrow develop into functional cells such as neutrophils and lymphocytes (4–7). Undoubtedly as a consequence of its ability to increase the rate of development of cells involved in the immune response, treatment with leucogenenol enhances the immunocompetence of an animal and increases the rate at which immunosuppressed animals recover immunocompetence (8–13). Leucogenenol is also found as a thymo-thyroid hormone (14–16).




*0022-3549/84/1200-1846$01.00/0*

Having established that the effect of the mixture of metabolic products of *P. gilmanii* on circulating leukocytes is due to a single compound, leucogenenol, we turned our attention to identifying the compound(s) that induce a rise in rectal temperature of rabbits.

Raistrick and Rice (17) reported the isolation of 2,3-dihydro-3,6-dihydroxy-2-methyl-4-pyrone and curvularin from those metabolic products of *P. gilmanii* that are extractable from the culture medium by ether. Therefore, either or both of these compounds could be responsible for the temperature rise observed when the mixture of compounds extracted from the culture medium with ether is injected intravenously into rabbits (1). Accordingly, rabbits were treated with 2,3-dihydro-3,6-dihydroxy-2-methyl-4-pyrone and with curvularin (I) in quantities up to 100 μg/kg, and the effect on rabbit rectal temperature was measured. Neither compound had any effect; therefore, it was apparent that a hitherto unrecognized compound(s) was present in the ether-extractable metabolic products.



I



II

## RESULTS AND DISCUSSION

Preparative TLC following column chromatography of the ether-extractable metabolic products afforded curvularin (I), $C_{16}H_{20}O_5$, and a compound, $C_{16}H_{22}O_5$, in yields of ~35 mg and ~3 mg, respectively, from 1800 mL of culture medium. The compound $C_{16}H_{22}O_5$ induced a rise in the rectal temperature of rabbits when injected 1-10 μg/kg iv. Individually, or as a mixture, the other ether-extractable compounds did not affect rabbit rectal temperature.

The difference in molecular formula of $C_{16}H_{22}O_5$ from that of curvularin ($C_{16}H_{20}O_5$) and the similarity of their IR spectra suggested that $C_{16}H_{22}O_5$ resulted from the reduction of curvularin (I) to dihydrocurvularin (II). Accordingly, curvularin was reduced with sodium borohydride to yield two isomers of dihydrocurvularin ($R_f$ 0.4 and 0.6, EtOAc) one of which ($R_f$ 0.4) was found to be identical with that isolated from the metabolic products of *P. gilmanii*. It was not possible to identify the configuration of the isomers. Injection of either isomer of dihydrocurvularin induces a rise in the rectal temperature of rabbits. The naturally occurring isomer of $R_f$ 0.4 was used for further biological studies.

Figure 1 shows the effect of the injection of 3 μg/kg iv of dihydrocurvularin ($R_f$ 0.4) on the rectal temperature of rabbits. Unlike lipopolysaccharides, repeated dihydrocurvularin injections produce a rise in rectal temperature indistinguishable from that observed on initial injection (Fig. 1). The time required for the rabbit to show a maximum rise in rectal temperature varies with the animal. In a group of 12 rabbits, 8 showed a maximum rise in rectal temperature at ~6 h while 4 rabbits showed a maximum rise at ~2 h. No significant differences in the degree or time of temperature rise were observed when 1, 3, or 10 μg/kg of dihydrocurvularin was injected in groups of rabbits. Injection of 0.1 μg/kg of dihydrocurvularin had no significant effect on rectal temperature.

There are no significant changes in differential blood cell counts made at half-hour intervals for 24 h following treatment with dihydrocurvularin, nor do the rabbits show a leukocytosis or change in red blood cell counts in their peripheral circulation.

For some unexplained reason, previous treatment with lipopolysaccharide prevents a rise in temperature following injection of dihydrocurvularin. Since rabbits respond to treatment with dihydrocurvularin with either an immediate or delayed temperature rise, it was considered important to consider the two groups separately. Accordingly, rabbits were first treated with dihydrocurvularin, then with lipopolysaccharide, and then with dihydrocurvularin. None of the rabbits that responded with either an immediate or delayed temperature response responded to treatment with dihydrocurvularin 2 d or 1 or 2 weeks following their treatment with lipopolysaccharide. All the rabbits showed a maximum increase in rectal temperature 6-8 h after their initial injection with

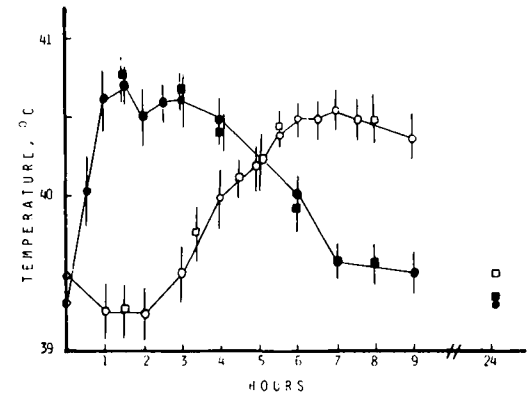



**Figure 1**—*Effect of the intravenous injection of dihydrocurvularin on the rectal temperature of rabbits injected with 3 μg/kg iv of dihydrocurvularin dissolved in pyrogen-free isotonic saline at a concentration of 10 μg/mL. Key: (●) initial injection; (■) reinjected 48 h later; (○) initial injection; (□) reinjected 7 d later. Vertical bars represent* SD. *Animals that responded with an increase in rectal temperature in 2 h (n = 4) were considered as one group; the remaining animals (n = 8) were considered as a second group. Before treatment rabbits showed a mean diurnal variation of 0.2 ± 0.1°C.*

lipopolysaccharide as well as the expected leukocytosis (18). Treatment with dihydrocurvularin had no significant effect on the response to lipopolysaccharide. On the other hand, none of the rabbits that were treated again with lipopolysaccharide 1 or 2 weeks following their initial injection showed a significant increase in rectal temperture.

It is possible that dihydrocurvularin could be of considerable use for investigating the effect of increased temperatures on physiological responses, as a tool to elucidate the mechanism of temperature regulation, as an important tool to study the role of fever (possibly isolated from other effects of leukocyte endogenous mediator) on the pathogenesis of infection, and possibly as a therapeutic agent.

## EXPERIMENTAL SECTION[1]

Solvents and other chemicals were redistilled or recrystallized as appropriate before use. Evaporation of solutions to lesser volumes or to dryness were carried out under reduced pressure in a rotary evaporator at 40-50°C (bath temperature). Where indicated, solutions were filtered under suction through a medium-porosity fritted glass Buchner funnel. Compounds were recrystallized until they showed a single peak on GC.

Rectal temperatures were taken with a telethermometer[2] and pyrogen-free isotonic saline solutions[3] were used for injection in the marginal ear vein of rabbits. Smears of peripheral blood, obtained from the marginal ear vein opposite the one used for injection, were made and the cells examined and enumerated in the usual manner. White blood cells and red blood cells were counted with a Coulter counter[4].

Groups of six rabbits (New Zealand White) (~2.5 kg) were used to determine the ability of each compound or mixture of compounds to induce hyperpyrexia. Prior to treatment rabbits showed a mean diurnal variation of 0.2 ± 0.1°C. The rabbit was weighed and then injected intravenously in the marginal ear vein with the calculated quantity (usually 1-10 μg) of the metabolic compound or compounds dissolved in 1-5 mL of pyrogen-free isotonic saline. Rectal temperatures were recorded at half-hour intervals for 12 h and again at 24 h. A rise of temperature of 1°C was considered significant. Standard deviations were not calculated for the experiments that led to the isolation of the compound that induces a temperature rise in rabbits. However, if one or more rabbits failed to respond with a significant rise in temperature, the experiment was repeated using greater quantities (≤100 μg) of the compound or mixture of compounds being investigated.

[1] Melting points were determined with a Kofler micro hot stage apparatus. UV spectra were determined with a Perkin-Elmer 323 spectrophotometer. IR spectra were determined with the Perkin-Elmer 727 spectrometer, and optical rotations were measured with a Perkin-Elmer 141 spectrometer using a 1-dm tube holding approximately 1 mL. GC were obtained on a Perkin-Elmer 900 gas chromatograph programmed from 200°C to 300°C at 12°C/min, using a flame detector and a column (30.48 cm × 2 mm i.d.) packed with 1% OV-17 on Gas-Chrom Q (18-100 mesh). Florisil (80-100 mesh) obtained from Fisher Scientific Co., Fair Lawn, N.J. was used for column chromatography and precoated silica gel plates (2000 μm, 20 × 20 cm), made fluorescent by the addition of zinc silicate (Anal Tech., Newark, Del.), were used for preparative TLC with the indicated solvents.

[2] Model 43; Yellow Springs Instrument Co., Yellow Springs, Ohio.

[3] Cutter Laboratories, Berkeley, Calif.

[4] Model F.

**Isolation of Curvularin and Dihydrocurvularin**—*Penicillium gilmanii* was grown as previously reported (1) at room temperature on Czapek Dox medium containing added zinc and copper ions (19) for ~6 weeks. The medium (1800 mL) then was separated from the mycelia by filtration through fluted filter paper[5] and continuously extracted[6] with ether (500 mL) for ~8 h. The ether extract was dried over anhydrous sodium sulfate, filtered, and evaporated to ~25 mL. The solution was then allowed to flow under gravity through a column (15 × 2 cm) of Florisil previously washed with absolute ethanol (500 mL) and ether (500 mL) and still wet with ether. An additional 200 mL of anhydrous ether was allowed to flow through the column followed by 100 mL of ethyl acetate. The ether and ethyl acetate that had flowed through the column was evaporated to dryness. The combined weight was ~1 g. The dry residue was dissolved in 10 mL of isotonic saline and ~1 mL/kg was injected into each of six rabbits. There was no significant rise in the rectal temperature of the rabbits.

The column was then eluted with dioxane (250 mL) which was evaporated to dryness and separated into four fractions by preparative TLC using ethyl acetate as the developing agent. Zones were made visible under UV light. Each zone ($R_f$ 0.8, 0.45, 0.1, and 0.0) was scraped off the plate and its contents eluted with dioxane.

The contents of the zone with $R_f$ 0.8 on evaporation to dryness appeared as a crystalline mass (35 mg) which on recrystallization from ether or ethyl acetate yielded ~30 mg of curvularin as white needles, mp 209°C [lit. (17) mp 205°C]. There was no depression in melting point on admixture with authentic curvularin. Curvularin sublimed unchanged at 140°C (bath temperature) under reduced pressure (0.05 mm Hg); $[\alpha]_{589}^{22}$ −35°, $[\alpha]_{578}^{22}$ −37°, $[\alpha]_{546}^{22}$ −45°, $[\alpha]_{436}^{22}$ −128°, $[\alpha]_{365}^{22}$ −240° (*c*, 1, EtOH); $\lambda_{max}$(EtOH): 273 nm (ε 8000) and 303 nm (ε 7100). The MS[13] (70 eV) showed $M^+$ 292.

*Anal.*—Calc. for $C_{16}H_{20}O_5$: C, 65.7; H, 6.9. Found: C, 65.8; H, 6.9.

The contents of the zone with $R_f$ 0.45 on evaporation to dryness yielded a syrup (3 mg) which crystallized from dichloromethane to give white needles (~2 mg), mp 175°C; $[\alpha]_{589}^{22}$ −38°, $[\alpha]_{546}^{22}$ −44°, $[\alpha]_{436}^{22}$ −64°, $[\alpha]_{365}^{22}$ −61° (*c*, 1, EtOH); $\lambda_{max}$ (EtOH): 284 nm (ε 2800); IR (KBr) $\nu_{max}$: 3440 s, 3390 s, 3260 m, 2970 m, 2950 s, 2930 s, 2910 s, 2850 m, 1740 s, 1630 s, and 1460 s $cm^{-1}$. The MS[7] (70 eV) showed $M^+$ 294 with clusters of major peaks at 276, 180, 161, and 147; CI − $NH_3$: $M^+$ 294.

*Anal.*—Calc. for $C_{16}H_{22}O_5$: C, 65.3; H, 7.5. Found: C, 65.3; H, 7.5.

Intravenous injection of the contents of the zone with $R_f$ 0.45 induced a febrile response. On elution and evaporation to dryness zones with $R_f$ 0.1 and 0.0 yielded syrups. Injection of rabbits with either fraction (1–20 μg/kg iv) did not induce a febrile response. The fractions were not investigated further.

**Sodium Borohydride Reduction of Curvularin to Dihydrocurvularin**—An excess (25 mg) of sodium borohydride was added to a cooled (ice water) solution of 50 mg of curvularin in absolute ethanol (5 mL) and set aside at room temperature. Water (2.5 mL) and acetic acid (2.5 mL) then was added and after standing at room temperature for several hours, the solution was evaporated to dryness and the solid residue was extracted with dioxane (5 × 5 mL). The dioxane extract was filtered and evaporated to dryness to yield a thick syrup, which on preparative TLC (ethyl acetate) showed two compounds with $R_f$ 0.45 and 0.65, which were scraped off the plate and eluted with dioxane.

Evaporation of the eluate of the zone with $R_f$ 0.45 yielded dihydrocurvularin (II) as a syrup (20 mg) which crystallized from dichloromethane (~15 mg), mp 175°C, which showed no depression on admixture with the naturally occurring compound of the same $R_f$ given above. The optical rotation, IR spectrum, UV spectrum, and MS were identical with those of the naturally occurring dihydrocurvularin of $R_f$ 0.45.

*Anal.*—Calc. for $C_{16}H_{22}O_5$: C, 65.3; H, 7.5; $M^+$, 294. Found: C, 65.3; H, 7.5; $M^+$, 294.

Evaporation of the eluate of the zone with $R_f$ 0.65 afforded isodihydrocurvularin as a crystalline mass of needles (30 mg), which on recrystallization from ethyl acetate (weight ~20 mg) had mp 186°C. The melting point was depressed on admixture with dihydrocurvularin. $[\alpha]_{589}^{22}$ −40°, $[\alpha]_{578}^{22}$ −41°, $[\alpha]_{546}^{22}$ −45°, $[\alpha]_{436}^{22}$ −66°, $[\alpha]_{365}^{22}$ −63°, (*c*, 1, EtOH). The IR and UV spectra were essentially indistinguishable from the corresponding spectra of dihydrocurvularin. Intravenous injection into rabbits induced a febrile response. The compound was not investigated further.

**Effect of Dihydrocurvularin on Rectal Temperatures and Circulating Blood Cells**—Twelve rabbits (weight, ~2.5 kg) selected at random from 50 animals were injected in the marginal ear vein with 3 μg/kg iv of synthetic dihydrocurvularin dissolved (10 μg/mL) in pyrogen-free isotonic saline. At half-hour intervals thereafter rectal temperatures were recorded and, in addition, a sample of blood (~0.4 mL) was taken from the marginal ear vein opposite to that used for injection. Smears of the blood were made on microscope slides, stained with Wright's stain, and differential counts were made in the usual manner. Additionally, the concentration of white and red blood cells was determined with a Coulter counter. Means and standard deviations were calculated from the results obtained from 4 of the 12 rabbits whose rectal temperatures rose within 2 h; means and standard deviations were also calculated from results obtained from the remaining 8 rabbits. The experiment was repeated using 0.1, 1, 3, and 10 μg/kg of dihydrocurvularin.

**Effect on the Temperature Response of Repeated Treatment with Dihydrocurvularin**—Each of the 12 rabbits that were injected with 3 μg/kg of dihydrocurvularin were reinjected with 3 μg/kg of dihydrocurvularin 24 h later, and their rectal temperatures were recorded at half-hour intervals for 12 h. At 2-d intervals for 2 weeks, each rabbit was reinjected with 3 μg/kg of dihydrocurvularin, and again their rectal temperatures were recorded at half-hour intervals for 12 h.

**Effect of Lipopolysaccharide on the Temperature Rise Induced by Dihydrocurvularin**—Each of six rabbits (group I) was injected with 3 μg/kg iv of dihydrocurvularin, and their rectal temperatures were measured at half-hour intervals for 12 h and again at 24 h as described above. Two days later each rabbit, and each of six additional rabbits (group II), received 1 μg/kg iv of lipopolysaccharide (*Escherichia coli* preparation, L-3129[8]), and their rectal temperatures were again recorded. Two days following their treatment with lipopolysaccharide each rabbit in group I was injected with 3 μg/kg iv of dihydrocurvularin and again their rectal temperatures were recorded at half-hour intervals as described. One week and 2 weeks later each rabbit in group I received 3 μg/kg iv of dihydrocurvularin, and each rabbit in group II received 1 μg/kg of lipopolysaccharide. Rectal temperatures were again recorded. Differential and white blood cell counts were made only on the rabbits that were treated with lipopolysaccharide.

## REFERENCES


(1) F. A. H. Rice, *Proc. Soc. Exp. Biol. Med.*, **123,** 189 (1966).

(2) F. A. H. Rice, *J. Chem. Soc.*, **1971,** 2599.

(3) F. A. H. Rice, *J. Infect. Dis.*, **118,** 76 (1968).

(4) F. A. H. Rice and J. H. Darden, *J. Infect. Dis.*, **118,** 289 (1968).

(5) F. A. H. Rice, J. Connolly, K. Aziz, and J. D. McCurdy, *J. Infect. Dis.*, **123,** 117 (1971).

(6) F. A. H. Rice, J. D. McCurdy, and K. Aziz, *Proc. Soc. Exp. Biol. Med.*, **136,** 56 (1971).

(7) G. C. Fossati, D. Fumarola, E. Cerra, and E. Cavalieri, *Riv. Emoter. Immunoematol.*, **16,** 91 (1969).

(8) F. A. H. Rice and R. Ciavarra, *Proc. Soc. Exp. Biol. Med.*, **137,** 567 (1971).

(9) F. A. H. Rice, R. Ciavarra, and T. Borsos, *Proc. Soc. Exp. Biol. Med.*, **140,** 471 (1972).

(10) F. A. H. Rice, N. D. Das, and M-J. Koo, *Proc. Soc. Exp. Biol. Med.*, **141,** 222 (1972).

(11) F. A. H. Rice and M-J. Koo, *J. Immunol.*, **127,** 1601 (1981).

(12) F. A. H. Rice, J. Lepick, and P. Hepner, *Radiat. Res.*, **42,** 164 (1970).

(13) F. A. H. Rice and J. D. McCurdy, *J. Immunol.*, **128,** 1769 (1982).

(14) F. A. H. Rice and B. Shaikh, *Biochem. J.*, **116,** 709 (1970).

(15) F. A. H. Rice, B. Shaikh, and C. G. Chen, *Johns Hopkins Med. J.*, **135,** 336 (1974).

(16) F. A. H. Rice, J. D. McCurdy, and C. Oresajo, *Am. J. Physiol.*, **238,** E540 (1980).

(17) H. Raistrick and F. A. H. Rice, *J. Chem. Soc.*, **1971,** 3069.

(18) E. G. Young and F. A. H. Rice, *J. Lab. Clin. Med.*, **29,** 735 (1944).

(19) G. Smith, *Trans. Br. Mycol. Soc.*, **32,** 280 (1949).


[5] Whatman No. 1.

[6] Soxlet.

[7] LKB 9000.

[8] Sigma Chemical Co., St. Louis, Mo.