As Dr. Provasoli says, let us "hope that the few men of good will will succeed to make fully understood that our safety is only in a united world policy and in the strong and truthful building of an organization governing the entire world.—*William Trager* (Rockefeller Institute, Princeton, New Jersey).

Prof. C. Bonne, head of the Department of Pathology in the Government Medical School in Batavia, has written to Dr. Morris E. Dailey, University of California Hospital, San Francisco. Portions of his letter, dated 12 February 1946, follow:

The European staff members of the School have all been interned by the Japanese, most of them, including myself, for three and one-half years. I am recuperating now in Australia with a few of my colleagues; others have gone to Holland and others are still in Java. Many of us have been severely ill, but there were no deaths amongst us. In January this year the Medical School was still in the hands of the Indonesians. School and hospital did not suffer much damage mainly due to the wise cares of our Indonesian assistants. I hope to be able to resume my work in my old position when a settlement between the Dutch and Indonesians is reached.

Prof. Bonne's present address is: c/o Mrs. J. Bonne, Main Avenue, Coorparoo, Brisbane, Queensland, Australia.

An international conference, called by the executive committee of the International Astronomical Union, met in Copenhagen on 7–11 March. The American delegation—Harlow Shapley, director of the Harvard University Observatory; Otto Struve, director of the Yerkes Observatory of the University of Chicago and of the McDonald Observatory of the University of Texas; and Joel Stebbins, director of the Washburn Observatory of the University of Wisconsin and a research associate of the Mount Wilson Observatory at Pasadena, California—left the United States on 2 March, flying from La Guardia Field.

The more precise determination of star positions was one of the matters considered at Copenhagen. This conference is expected to redistribute international services that were assigned to Germany, wholly in German hands, for the interwar period. It is likely that Russia will take over one or two of these service bureaus, which deal with planetary motions, with variable stars, and with the international time services.

In the Laboratory

Inhibition of Fungus Respiration: a Metabolic Bio-assay Method [1]

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In the search for chemical substances active against disease-producing organisms, one is frequently confronted with difficulties in establishing criteria for activity. In a stimulating series of papers on the principles and practices of laboratory testing of fungicides, McCallan and Wilcoxon (*7*) bring out the close relationship of bio-assay methods in general and speak of two categories of assays: (1) that in which the effect of a toxic agent on an organism is *measured* quantitatively, and (2) that in which some phenomenon in the form of a "response" (an event either does or does not happen) of an organism is *observed.* Nearly all bio-assay methods for fungicides and bacteriocides in current use are examples of Type 2. Among these may be mentioned the various spore-germination tests and the U. S. Food and Drug Administration methods (*11*) for testing antiseptics, wherein the highest dilution of a chemical completely inhibiting growth is observed. The agar cup-plate methods, utilized in penicillin assay, are further examples, since one determines essentially a zone of concentration which inhibits growth.

While observations of certain responses of an organism, *i.e.* the germination or nongermination of spores, growth or absence of growth, have provided much valuable information on antiseptics, disinfectants, and substances with chemotherapeutic activity, need is felt at times for methods that will reveal the extent to which a foreign agent affects a healthy, growing culture. This is particularly true with the pathogenic fungi. We know of chemicals that keep spores from germinating or prevent fungus mycelia from entering a zone of given concentration of chemical on an agar plate. However, in spite of several studies on the correlation between laboratory and clinical findings, our information on what (to say nothing of the mechanism whereby) the chemicals do to "adult" fungi is extremely scanty. In the treat-

[1] The author appreciates the interest and aid of Dr. J. G. Hopkins, Lt.Col. Laurence Irving, and Lt.Col. J. R. Scholtz in the work reported here and to be described.

ment of fungus infections we are primarily concerned with the removal of an established parasite and secondarily with prevention of reinfection. Information from "response" tests is directly applicable to considerations of reinfection but only indirectly to an existing infection. It is clear that all fungus infections are not acquired via the fungus spore; indeed, the mode of transmission of fungus infections is far from being clearly explained with some evidence incriminating mycelia contained in skin debris as the infecting agent. Though fungus spores are notoriously resistant, it does not follow that treatment which prevents their germination will also be lethal to mycelial fragments (capable of growth) contained in skin debris in which the protective action of tissue and other factors are possibly operative.

The present unsatisfactory state of treatment for fungus infections may, in part, reflect our lack of satisfactory methods for testing *in vitro* the effects of chemicals on the fungus in a state of organization comparable to that *in situ*.

In view of the foregoing, convenient methods furnishing quantitative data on the immediate effect of a chemical on a mycelial organization of a fungus would be welcome. It is shown that such may be found in quantitative measurements of oxygen consumption by fungus cultures or samples therefrom; the methods may be extended to fungus mycelia contained in skin debris.

Cultures of pathogenic fungi freshly isolated from foot lesions were identified following Emmons (*5*), and Conant, *et al.* (*2*). These cultures and cultures of fungi received from Duke University Medical School[2] were inoculated onto plates of Sabouraud's dextrose agar and Difco-cornmeal agar. Transfers were also inoculated into a liquid medium. The following organisms were used: *Trichophyton gypseum, T. rubrum,* and *Epidermophyton floccosum.*

The filamentous fungi grow in the form of a mat on the surface of agar media and, unless disturbed, grow similarly on the surface of liquid media. Attempts to harvest such growth and to homogenize it in some way (such as by sucking in and expelling from a syringe) for use as a suspension have been reported occasionally, but such techniques defeat the purpose; the mycelial components are usually ruptured and one most often obtains merely a spore suspension. Using a sterile cork borer of 15-mm. diameter, cylinders were cut from agar-plate cultures, two or four weeks old. The discs were placed in a respirometer in 1 cc. of liquid. It made no difference whether the disc was placed so that the mycelial side or the agar side was face down in the liquid. After the experimental period in the respirometer the disc was dropped into boiling water to remove the agar, lifted from the water after 1 minute, drained, and placed on a previously weighed, clean, dry cover slip. Dry-weight determinations were made by heating the fungus and cover slip for 12 hours at 100° C. Loss of weight by the fungus mycelium during the brief hot-water extraction was found to be negligible. After

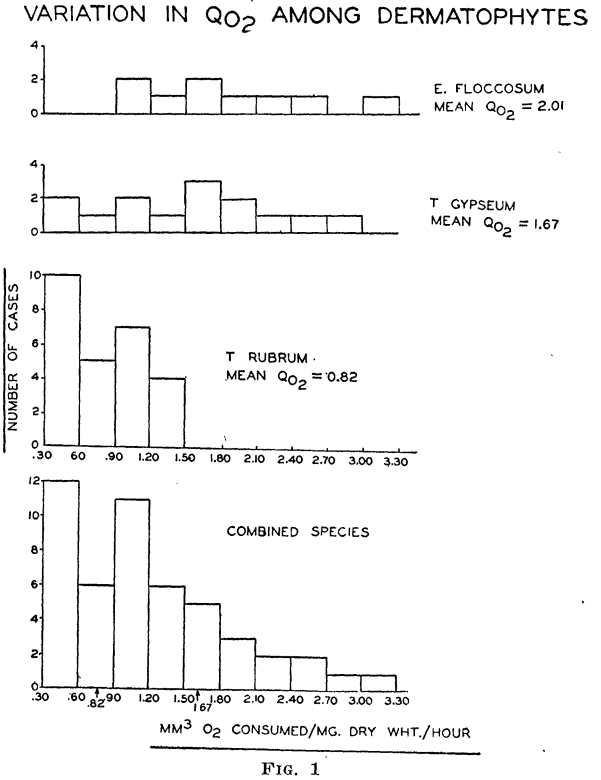

FIG. 1

the drying and weighing the cover slip and fungus were mounted, in lactophenol cotton blue, on a slide to give a permanent record of the organism used in each experiment. With this record, contamination, if such occurred, would be easily discovered. Samples of fungus from liquid cultures were obtained simply by use of a wire, flattened at the end to a sharp cutting edge capable of being flamed. Pieces of mycelium were lifted out, drained, rinsed in sterile distilled water, and placed in 1 cc. of liquid in the respirometer vessel.

A volumetric microrespirometer described by Scholander and by Scholander and Edwards (*9*) was used in the present work.[3] This instrument, discussed by Glick (*6*), employs an ordinary micrometer to displace mercury from the reservoir of a Rehberg-type microburette and has a sensitivity of 1/3 mm.³/hour. An

[2] The kindness of Dr. N. F. Conant in supplying cultures of organisms is appreciated.

[3] It is a pleasure to acknowledge the helpful discussion with Major P. F. Scholander and Lt. G. A. Edwards during the course of this work.

important feature of the instrument is that the pressure of oxygen in the vessel is maintained constant during long experiments.

The influence of several classes of chemical substances under different environmental conditions on the oxygen consumption by species of dermatophytes will be reported elsewhere (*8*); a few examples will suffice here. Variation in the Q_{O_2} (mm.^{3} O_2 consumed/mg. dry weight/hour) among isolates of the three species examined is shown in Fig. 1. Four of the values given for *Trichophyton gypseum* are for oxygen consumption in distilled water; for the other

TABLE 1

EFFECT OF INORGANIC SALTS ON OXYGEN CONSUMPTION BY DERMATOPHYTES

Compound	Concentration	Organism	Change from Q_{O_2} in M/15 KH_2PO_4 buffer at pH 4.6
			%
$ZnCl_2$	10^{-2}M	*E. floccosum*	− 93
$ZnCl_2$	10^{-3}M	*T. rubrum*	− 32
$Zn(NO_3)_2$	10^{-3}M	"	− 22
$ZnCl_2$	10^{-4}M	*T. gypseum*	− 13
$CdCl_2$	10^{-2}M	*T. rubrum*	− 22
$CdSO_4$	10^{-2}M	"	+ 37
$AgNO_3$	10^{-2}M	"	−100
$AgNO_3$	10^{-4}M	"	− 43
$HgCl_2$	10^{-2}M	"	− 74
$HgCl_2$	10^{-3}M	"	− 28

two species all values were obtained in M/15 KH_2PO_4 buffer at pH 4.6; no substrate or other chemical was added in any case. After a preliminary equilibration period, the rate of oxygen consumption for all organisms examined was linear for several hours. The mean Q_{O_2} of 0.82 (26 cases) for *T. rubrum* sets it somewhat apart from the other species examined.

Certain inorganic water-soluble salts were examined for effect on fungus respiration. As will be seen in Table 1, mercury, silver, and zinc in similar concentrations depressed respiration to a comparable extent; cadmium compounds had little effect. Addition of 1/100 molar sodium propionate to *E. floccosum* in M/15 KH_2PO_4 buffer resulted in three experiments in inhibitions of 13, 27, and 35 per cent, respectively, of the basal Q_{O_2} in plain buffer. Removal of the propionate by washing with buffer and return of the fungus to buffer was followed in each of the three cases by a rise in the Q_{O_2} to the initial basal rate in plain buffer. Exposure of *E. floccosum* to 1/100 molar zinc chloride for three hours was not followed by recovery in the Q_{O_2} when the salt was removed; the fungus was incapable of further growth when transferred to an agar medium after such treatment.

Clinical trials with dilute (1 per cent) solutions of zinc chloride showed it to be of promise in the treatment of tinea cruris and tinea glabrosa. A report on this work is in preparation (*3*).

The use of respiration studies in connection with problems of skin infection has been advised before. Bronfenbrenner, Hershey, and Doubly (*1*) and Ely (*4*) advocated use of manometric methods for evaluation of germicides, but few publications have appeared in this vein. It is believed from the present study that the uncovering and evaluation of substances for chemotherapeutic use in fungus infections by determination of their effect on fungus respiration is a practical and desirable procedure. Chemical substances proposed as fungicidal or fungistatic agents can easily be subjected to critical test through measurement of inhibitory action on fungus respiration.

Though the metabolism of the pathogenic fungi is practically unknown, it is realized that inhibition of oxygen consumption may be an insignificant matter with some species if the organism in question happens to employ hydrogen acceptors other than oxygen for the bulk of its metabolism. Sevag, Richardson, and Henry (*10*) have stated this problem very clearly in their studies on the mode of action of sulfonamides on the respiration of bacteria. With the species herein discussed, oxygen consumption is apparently a critical index of metabolism and inhibition of respiration a valuable *in vitro* criterion of the effect of chemotherapeutic agents on these organisms.

The application of the method presented is not limited to the dermatophytes. One can apply it to the fungi causing deterioration of textiles and other equipment in wet tropical climates. A small sample of cloth infected with an assortment of organisms may be placed in a respirometer and the oxygen consumption of this *microcosm* measured. Various chemicals may then be added and their comparative inhibitory effect on respiration noted. This procedure would enable one to determine the effect of treatment on a mixed population in which synergistic phenomena are doubtless in operation.

In actuality, any situation in which mycelial growth of fungi needs to be controlled could profit from the application of metabolic criteria to the activity of a fungicidal or fungistatic agent.

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