types of mice (*15*). However, the possibility of dilution of the radioactive compound by endogenous substrates was not taken into account in these experiments. A higher rate of fatty acid synthesis in obese mice as compared to their nonobese littermates has so far been demonstrated only in vivo (*16*).

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27 February 1964

Sterol Induction of Reproduction and Stimulation of Growth of Pythium and Phytophthora

Abstract. *Sterols of fungal, plant, and animal origin induced sexual reproduction in* Pythium periplocum *and* Phytophthora megasperma *var.* sojae *and the formation of large zoosporangia in* Phytophthora parasitica *var.* nicotianae. *Some sterols, especially cholesterol, stimulated growth.*

Although considerable emphasis has been placed upon carbohydrate, nitrogen, and water-soluble vitamin nutrition of species of *Phytophthora* and other Phycomycetes which cause plant diseases, lipid nutrition has received little attention. Recently, vegetable oils were found to be better carbon sources than glucose for *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker (*1*). Apparently, non-saponifiable compounds were involved (*2*). Triolein was a poor carbon source, and charcoal-treated oat oil supported less growth than untreated oat oil. Cholesterol and tocopherol reversed the toxicity of oleic acid. Cholesterol and a fatty acid fraction of cow liver stimulated the growth of *P. infestans* de Bary (*3*).

When *P. parasitica* var. *nicotianae* was grown on a vegetable oil-nitrate medium, zoosporangia were numerous in and near the densely colonized oil droplets (*1*, *2*). No reports of reproduction of species of *Pythium* or *Phytophthora* on defined media were found. These observations led to the studies described here of the effects of sterols on growth and reproduction of species of Pythiaceae.

Cultures used were *Pythium periplocum* (Drechs.), *Phytophthora megasperma* (Drechs.) var. *sojae* (Hilde.), and *P. parasitica* var. *nicotianae* (*4*). The medium contained 5.4 g glucose, 1.5 g $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 ml of a 1000-ppm stock solution of thiamine-HCl, 17 g agar, and 1000 ml distilled water. The *p*H was adjusted to 6.0 before the medium was autoclaved for 10 minutes. Solutions of sterols in ether (*5*) were applied to the surface of the solidified agar (25 ml/plate) at the rate of 20 mg of sterol per liter of medium. Control plates received an equal volume (2 ml) of ether reagent. The plates were seeded several hours after the ether solutions were applied. The inoculum was produced by transferring mycelium from the stock culture (maintained on potato-dextrose agar) to 1.7 percent agar in petri plates. Disks cut with a No. 1 cork borer at the edge of the resulting colony were transferred to the edge of petri plates containing the glucose-nitrate medium. Plates were incubated in the dark at about 25°C. Linear growth was measured 3 and 7 days after seeding the *Pythium* and the *Phytophthora* isolates, respectively. The plates were examined microscopically for reproduction 10 days after seeding.

Oospores of *Pythium periplocum* and *Phytophthora megasperma* var. *sojae* were abundant on sterol media. The controls were completely vegetative. *Phytophthora parasitica* var. *nicotianae* produced numerous large sporangia and chlamydospores in the presence of sterols. In the absence of sterols, small abnormally shaped sporangia and small chlamydospores were formed. Whether these abortive-appearing sporangia were functional in producing zoospores was not investigated. There were no obvious differences in the numbers of oospores or sporangia produced per unit area on the various sterol-containing media.

Sterols varied in their capacity to stimulate growth (Table 1). Cholesterol and phytosterol consistently stimulated growth. The purity of the sterols used (*6*) was not specified. Regardless of whether the differences in growth on media containing various sterols are due to impurities or to sterol structure, the inhibition or lack of stimulation of growth by some sterols argues against the concept (*7*) that there are no fungal requirements specifically for reproduction and independent of growth effects.

To determine whether the activity was due to an impurity, equivalent amounts of digitonin and a purified grade of cholesterol (99+ percent) (*8*) were mixed as 1 percent solutions in 95 percent ethanol. The digitonide was removed by filtration, washed

Table 1. Linear growth (in millimeters) of *Pythium periplocum* (Py.p.), *Phytophora megasperma* var. *Sojae* (Ph.m.), and *Ph. parasitica* var. *nicotianae* (Ph.p.) on glucose-nitrate agar medium supplemented with sterols (20 mg/liter).

Supplement	Py.p.*	Ph.m.†	Ph.p†
Ergosterol	39.5	12.0	11.0
Phytosterol	46.5	15.5	25.0
Stigmasterol	26.5	9.0	19.0
β-Sitosterol	28.0	7.0	17.5
Cholesterol	52.5	15.5	26.5
None	38.3	6.7	13.7
Statistical data			
LSD .05 ‡	7.8	3.3	3.1
LSD .01 ‡	11.1	4.7	4.5

* After 3 days' growth. † After 7 days' growth. ‡ Least Significant Difference at the 5 or 1 percent confidence level.

thoroughly with ether and 95 percent ethanol, and dissolved in pyridine. The digitonin was precipitated with ether and removed by centrifugation. The cholesterol was reduced to dryness in an air stream and dissolved in ether. The lower limits for activity were not investigated, but the digitonin-precipitated cholesterol induced oospore or sporangium formation at 0.2 mg/liter of medium. Digitonin alone was not active.

Leonian and Lilly (*9*) reported on a fat-soluble substance which induced oospore formation by species of *Pythium* and *Phytophthora*. They stated that the active substance was strongly adsorbed onto sterols, which, when crystallized repeatedly from hot ethanol, were inactive. The active substance distilled at 110° to 135°C at 0.1 mm-Hg and was active at 0.1 to 1 part per million. The digitonin-precipitated cholesterol was active in the same concentration range; thus its reproduction-inducing activity is probably not due to an impurity. It would be of interest to determine whether Leonian and Lilly's substance was a sterol derivative or a different type of compound.

Exogenous sterols are required for the growth of certain protozoa, a slime mold, and pleuropneumonia-like organisms (*10*). A sterol was found to initiate the aggregation of amoebae of the slime mold *Dictyostelium discordeum* Raper to form a multicellular unit, the first step in reproduction. It appears likely that sterols participate directly or indirectly in reproduction of microorganisms, and that those organisms unable to synthesize sterols require an exogenous source.

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31 January 1964

Heart Rate Reactions and Locus of Stimulation within the Septal Area of the Rat

In the 14 June 1963 issue of *Science* there were two reports (*1*, *2*) dealing with heart rate changes accompanying brain stimulation in the rat. Both papers referred to my earlier report (*3*) of heart rate slowing associated with intracranial self-stimulation of the septal area in rats.

Perez-Cruet *et al.* (*2*) make the following statement. "Our findings on the slowing of heart rate during septal self-stimulation are in substantial agreement with Malmo's results despite the difference in methods of measurement in the two experiments." These workers determined mean heart rates for 10 to 14 periods of 5 minutes each, with brain stimulation and no brain stimulation alternating from one 5-minute period to the next; and they compared the mean heart rate for combined stimulation periods with the mean heart rate for combined nonstimulation periods. With my data, on the other hand, differences for single bar-presses were obtained by subtracting poststimulation heart rates from the prestimulation heart rates, and from these single difference values a mean difference was computed for each animal. Meyers *et al.* (*1*) in summarizing the results of their third study say, "the early effect of septal ICS is accelerative, but the late effect is pronouncedly decelerative." This conclusion was based on results from beat-by-beat measurements.

Although earlier I had not actually measured heart rate during the first second after stimulation, careful inspection of my tracings had not revealed this initial accelerative phase observed by Meyers *et al.*, and this impression gained from inspection was later confirmed by careful beat-by-beat analyses on six animals in my most recent ICSS experiment (*4*, p. 8). These animals were selected for measurement because they were the only ones with satisfactory respiration tracings recorded along with heart rate during septal self-stimulation.

The results of the beat-by-beat measurements for these six subjects are shown in Fig. 1. In no case did the heart rate for any of the first three poststimulation points on the curves exceed the modal level of the prestimulation curve. All curves show an initial drop in heart rate followed by what appears to be a compensatory rise (roughly in proportion to the degree of initial fall) with another fall, more prolonged than the initial one, coming afterward. Only in one case (subject 16) did the rising phase reach the level of the prestimulation curve. In this subject, the rising phase actually exceeded the prestimulation level at two points; but the falling phase after this rise was extremely marked, and the overall main effect was clearly one of heart rate slowing. In short, in no case was initial heart rate acceleration observed.

Clearly, the problem was to discover the reason for the different findings in the two laboratories; and after an exchange of detailed histological findings, it now appears very probable that the different results were obtained because there was a consistent dif-

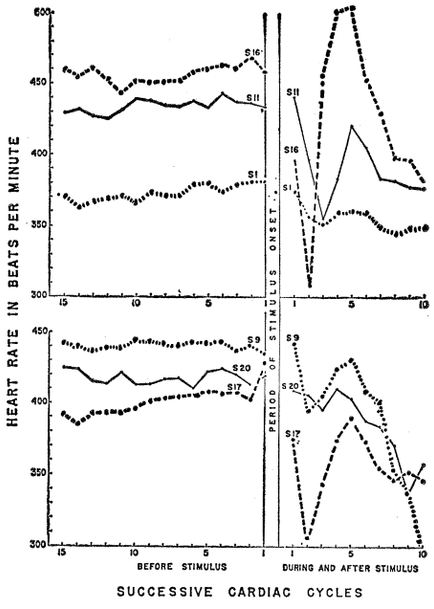

Fig. 1. Temporal course of cardiac response. Data are from six animals in experiment 2 (*4*, p. 8). Averages are based on all available data for these animals (that is, all artifact-free tracings for bar-presses during five 10-minute sessions and one 5-minute session, in which there had been at least 8 seconds between the beginning of the stimulation in question and the termination of the immediately preceding stimulation). Period of stimulus onset: the one cardiac cycle (that is, the distance between the peaks of two successive QRS waves) in which onset of 0.5-second septal stimulation occurred. Since this cycle was a mixture of pre- and poststimulation, it was eliminated from measurement.