about 4 minutes after a supercooled cloud was seeded.

Besides using dry ice as a source of ice nuclei, a rod cooled in liquid air and passed rapidly through the supercooled cloud leaves a trail containing great numbers of submicroscopic nuclei which, due to microturbulence, spread through the cloud, causing it to dry up as the ice crystals grow. Subsequent experiments show that myriads of ice nuclei form spontaneously if a copper rod having a temperature of −35° C. is placed in a supercooled cloud having a temperature of −12° C. When replicas are made of the nuclei, which stream from the copper rod, they are found to have dimensions of less than 1 μ. Some of these tiny crystals show the trigonal symmetry of crystalline ice and are thin, triangular prisms.

Experiments are under way to investigate various aspects of this spontaneous development of ice crystals in order to determine whether relationships can be established between the laboratory experiments and the natural atmosphere.

It is planned to attempt in the near future a large-scale conversion of supercooled clouds in the atmosphere to ice crystal clouds, by scattering small fragments of dry ice into the cloud from a plane. It is believed that such an operation is practical and economically feasible and that extensive cloud systems can be modified in this way.

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Technical Papers

Ergosterol From the Mycelium of *Penicillium notatum* (Submerged Culture)

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The occurrence of ergosterol in the mycelia of molds has been known for some time, and its presence in the mycelia of various surface-cultured *Penicillia* has been recorded (*2, 4, 5*). Later, Whitmore, *et al.* (*6*) found ergosterol to be present in the mycelium of *P. notatum*. Cavallito (*3*) confirmed this and isolated it from the mycelia of *P. chrysogenum* and *P. citrinum*. The latter showed that in the mycelia of *P. notatum*, grown by submerged fermentation, traces of ergosterol could be detected spectrographically but not in quantities which could be isolated.

The present authors have studied the sterol fraction from a strain of *P. notatum* (X-1612) grown by submerged culture and have isolated and identified ergosterol by means of its physical characteristics and by those of the benzoate.

The residue resulting from the evaporation of an alcoholic extract of 2.88 kg. of air-dried mycelium (obtained from a production run) was extracted with ether. Concentration of the ether extract (after drying over sodium sulfate) gave an oil weighing 145 grams, which by photocolorimetric determination (*1*) showed an ergosterol content of 4.4 per cent; this was in agreement with ultraviolet absorption spectra data (Fig. 1). Saponification gave a crude sterol fraction weighing 8.58 grams and containing about 45 per cent ergosterol by both methods of determination. The absorption spectrum of this fraction is shown in Fig. 1.

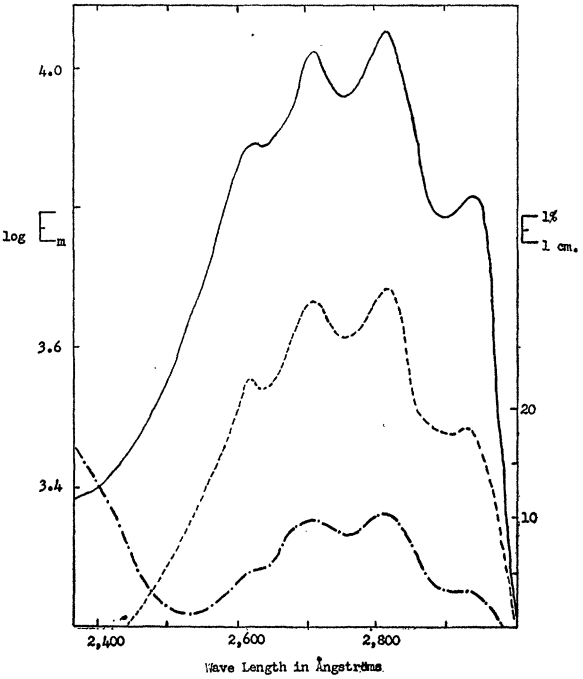

FIG. 1. Ultraviolet absorption curves of residue from alcohol-ether extracts, —·—·, calculated as $E^{1\%}_{1cm.}$; crude sterol fraction, - - - - -, calculated as E_m; purified ergosterol from mold, ———, calculated as E_m.

Recrystallization of the sterol fraction from alcohol-benzene (2:1) gave 4.52 grams of crude ergosterol—m.p., 124–133° C.—which, after further purification by fractional crystallization, yielded ergosterol

—m.p., 157.5–160° (corr.), not lowered by mixing with an authentic sample of ergosterol;[1] $[\alpha]^{25}_D$, −128.6; E_m, 11,160 (2,820 A.) (Fig. 1). The benzoate was prepared—m.p., 169.5–171° (corr.), not lowered when mixed with an authentic sample of ergosterol benzoate;[2] $[\alpha]^{25}_D$, −67.2.

Ergosterol has been detected in, and isolated in quantity from, the mycelium of *P. notatum* (X-1612) when the mold is grown in submerged culture.

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The Effect on the Chick Embryo of the Simultaneous Inoculation of Stool, Streptomycin, and Penicillin

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Developing chick embryos were used in an attempt to isolate a virus from the stools of patients with epidemic diarrhea, nausea, and vomiting (*6*) (viral dysentery). To attempt to decrease the loss of virus due to filtration and yet eliminate bacterial growth, unfiltered stool suspensions were combined with penicillin, streptomycin, or combinations of both before inoculation of the embryo. Parker and Diefendorf (*5*) showed that the injection into chick embryos of 50–1,300 units of penicillin had no observable effect on the growth of the viruses of vaccinia, St. Louis encephalitis, or equine encephalomyelitis. Hirst (*3*) was able to increase the frequency of growth of influenza virus in chick embryos by the addition of penicillin to unfiltered throat washings. The protection of chick embryos from fowl typhoid by the use of streptomycin has been described by Jones, *et al.* (*4*). Florman and his co-workers (*2*) demonstrated the ability of influenza virus to grow in the presence of streptomycin in concentrations up to 12,000 units per egg. There was no evidence of any lethal effect on the developing embryo. Reimann, *et al.* (*7*) have observed that in the human colon 600 μg. (units) of streptomycin per gram of feces may in some instances be sufficient to kill all the colon bacilli.

[1] The physical constants of ergosterol (hydrate) are: m.p., 160–163°; $[\alpha]^{20}_D$, −128.7 (K. C. Callow. *Biochem. J.*, 1931, **25**, 79); Em, 11,500, (2,810 A.) (W. Huber, G. W. Ewing, and J. Kriger. *J. Amer. chem. Soc.*, 1945, **67**, 609).

[2] The physical constants of ergosterol benzoate are: m.p., 168–170°; $[\alpha]^{25}_D$, −70.5 (H. Wieland, and M. Assano. *Ann.*, 1929, **473**, 300).

Method

Thirteen- or 14-day-old developing chick embryos were inoculated, by the intra-amniotic method of Burnet (*1*), with 0.25–0.30 cc. of inoculum. The eggs were sealed with cellophane tape and incubated at 37° C. for approximately 40 hours. (This time was established during the early part of the study after noting the peak of the death rate of test and control eggs.)

Three different stool specimens were used: two which had been preserved on dry ice for 1–3 months, taken from persons suffering from epidemic diarrhea, nausea, and vomiting, and one fresh "normal" specimen. Ten-per cent suspensions of stool in broth were centrifuged at 1,500 r.p.m. for 10 minutes and the supernatant material collected for use as inoculum.

The solution of penicillin was prepared by dissolving the sodium salt in sterile physiologic saline to a final dilution of 25–200 units in 0.05 cc. Streptomycin hydrochloride was dissolved in sterile physiologic saline to make a solution containing 5,000 μg. (units)/0.05 cc.

TABLE 1

Group	Stool suspension (cc.)	Saline (cc.)	Penicillin (cc.)	Streptomycin (cc.)	No. eggs	Embryos		
						Living	Dead	Positive cultures
1	0.2	0.05	...	...	20	0	20	20
	...	0.25	...	...	20	14	6	3
2	0.2	...	0.05	...	20	2	18	20
	...	0.2	0.05	...	20	16	4	0
3	0.2	...	...	0.05	15	4	11	1
	...	0.2	...	0.05	15	7	8	0
4	0.2	...	0.05	0.05	20	10	10	1
	...	0.2	0.05	0.05	20	11	9	1

Penicillin: 0.05 cc. (25–200 units); streptomycin: 0.05 cc. (5,000 μg. or units); stool suspension: 0.2 cc. (10-per cent suspension).

Each inoculum containing stool suspension was allowed to stand at room temperature for 30–90 minutes before injection. A similar group of controls was performed, substituting sterile physiologic saline (0.2 cc.) for the stool suspension. The different types of inocula are outlined in Table 1.

After about 40 hours each egg was opened aseptically, the appearance of the embryo noted, and fluid removed for culture. This material from each egg was incubated in a tube of hormone broth for approximately 24 hours; then blood agar plates were inocu-