Table 2. Effect of incubating several proteins with LDH-1, NAD, and NADH. The incubating mixtures contained: $3.5 \times 10^{-6}M$ LDH-1, 14.0 mM NAD, 1.4 mM NADH, and $7.0 \times 10^{-6}M$ G-3-PD or $3.5 \times 10^{-6}M$ MDH or 2.5 mg of BSA per milliliter, all made up in 0.05M tris-HCl buffer, pH 7.4. Final concentrations in the cuvette were: $1.75 \times 10^{-6}M$ LDH-1, 7.0 mM NAD, 0.7 mM NADH, $3.5 \times 10^{-6}M$ G-3-PD or $1.75 \times 10^{-6}M$ MDH or 1.25 mg of BSA per milliliter, and 0.5, 1.0, 5.0, and 10.0 mM pyruvate. Reactions were initiated by the addition of pyruvate, and, in the case of NAD alone (last column), by pyruvate and NADH.

Final pyruvate in cuvette (mM)	Incubation (min)	Percent initial LDH-1 activity remaining after incubating LDH-1 with:				
		NAD, NADH, and G-3-PD	NAD, NADH, and MDH	NAD, NADH, and BSA	NAD and NADH	NAD
0.5	10	100	100	100	75	51
	30	100	100	60	25	51
1.0	10	100	100	100	63	45
	30	100	100	57	20	47
5.0	10	100	100	85	25	44
	30	100	100	57	6	44
10.0	10	100	100	57	13	46
	30	100	85	43	3	45

ternary complex are incubated alone, inhibition is never complete (Table 1) because, when NADH is added to start the reaction, NADH begins to displace NAD from the complex. Hence, the ternary complex cannot stop the reaction completely. Table 1 shows that at physiologic enzyme concentrations LDH-1 (pig heart) is inhibited more than LDH-5 (rabbit muscle) when LDH, NAD, and pyruvate are incubated together without exposure to commonly occurring protective intracellular compounds. In previous experiments on ternary complex formation, we demonstrated that LDH-1 and LDH-5 from these species reacted similarly to homologous isozymes obtained from a single tissue (*1*). Table 1 also shows the considerable reduction in LDH-1 inhibition produced by glyceraldehyde 3-phosphate dehydrogenase (G-3-PD) (rabbit muscle) when G-3-PD is incubated in a mixture of LDH-1, pyruvate, and NAD. If another common intracellular enzyme, malate dehydrogenase (MDH) (pig heart), is substituted for G-3-PD, LDH inhibition is also decreased (Table 1). Reduced LDH inhibition presumably results from binding of NAD to MDH or G-3-PD. This binding decreases the extent of abortive ternary complex formation. Table 1 shows that bovine serum albumin (BSA) protected LDH-1 activity slightly.

Another important intracellular substance that can affect abortive ternary complex formation but ignored by the model that considers only incubation of LDH, NAD, and pyruvate is NADH. Table 2 shows that, when NAD and NADH are incubated with LDH, alteration occurs in the extent of LDH inhibition. Furthermore, incubation of either MDH or G-3-PD with NAD, NADH, and LDH almost completely eliminates LDH inhibition (Table 2). Under these conditions even BSA exerts a protective effect (Table 2).

These experiments show that such commonly occurring intracellular compounds as G-3-PD, MDH, or BSA reduce the extent of LDH inhibition and abortive ternary complex formation. Therefore, the relative availability of NAD to LDH as compared to other proteins appears to be an important factor determining the extent of abortive ternary complex formation and LDH inhibition. Since NAD and NADH concentrations change continuously within cells (*7*), their concentrations at actual sites of the LDH isozymes and other NAD-linked dehydrogenases are difficult to determine as are the extent of abortive ternary complex formation and LDH inhibition. However, as Coulson and Rabin suggest, LDH inhibition by pyruvate is attributable to the enol form present in commercial preparations (*2*); thus the actual extent of intracellular LDH inhibition would be restricted by the enol–keto tautomerization rate of pyruvate.

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Sexual Reproduction in Geotrichum candidum

Abstract. *A perfect state of* Geotrichum candidum *was isolated from soil in Puerto Rico. Wild-type cultures are self-fertile but give rise to self-sterile, cross-fertile mating types morphologically different in some respects from the wild type. This discovery of the perfect state of* G. candidum *and its unique pattern of sexuality may contribute to knowledge of its ecology, the origin of pathogenic races, and speciation.*

Geotrichum candidum Lk. *ex* Pers. is a versatile ubiquitous asexual fungus of considerable importance to man. It causes plant disease (*1*), contributes to slime accumulations in polluted streams (*2*) and in pulp and paper mills (*3*), and causes sludge bulking in disposal systems for human waste (*4*). It is associated with chronic bronchitis and intestinal disorders of man (*5*) and mycoses in animals (*6*). Few fungi are adapted to as many different habitats.

In an ecological study of *G. candidum* in soils of Puerto Rico, two self-fertile ascosporogenous cultures were isolated, each from soil supporting the growth of unidentified grasses. They are Pr-47 (30 July 1967) from the Sierra de Luquillo, at about 1500-foot (457-m) elevation on a north slope; and Pr-11 (26 June 1967) from near Maricao in West Central Puerto Rico from a south slope at about 2500-foot (761-m) elevation. The collection sites were in areas of high rainfall (100 inches or more per year) and probably

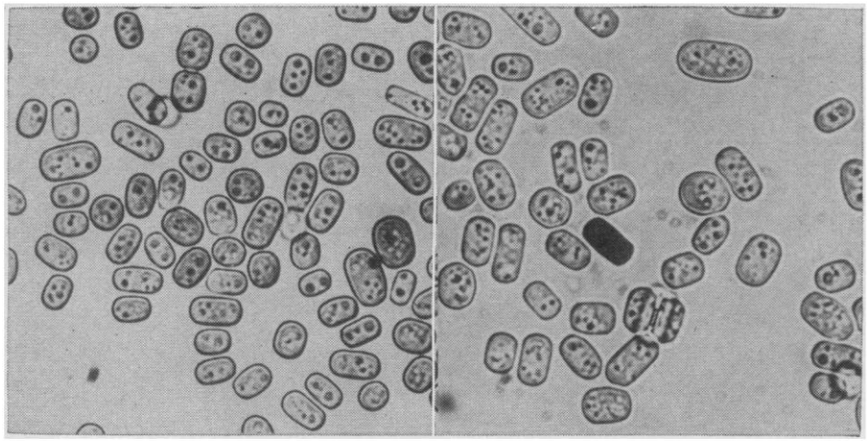

Fig. 1. *Geotrichum candidum* (×800). (Left) Typical arthrospores from a representative isolate from a worldwide collection. (Right) Arthrospores derived from a single ascospore of isolate PR-47. All cultures were grown simultaneously on potato-dextrose agar at 30°C for 24 hours and stained with crystal violet (200 μg/ml).

have no recent history of agricultural use.

On V-8 juice agar, malt extract, and most other natural media commonly used to culture fungi, 2-day-old cultures held at 25°C are typical of *Geotrichum candidum*, with aerial chains of arthrospores (Fig. 1), dichotomous hyphal branching at the periphery of the colony, and a fruity odor. Dominating the cultures in 3 to 5 days are hyaline oval to globose, punctate, median furrowed asci, each with a single smooth, thick-walled ascospore. The asci, formed only at septa, are preceded by the fusion of globose gametangia.

Although the wild-type cultures are self-fertile, compatible self-sterile clones were obtained as sectors and from conidia of the self-fertile wild type. Paired compatible self-sterile cultures always produced relatively long filamentous gametangia which fused in pairs at their apexes. Spore formation is analogous to that observed in species of *Rhizopus* in the Mucorales. Ascospores formed from the pairing of asexual Pr-47A_1 and Pr-47A_2 produced only the self-fertile Pr-47 type; Pr-11A_1 and Pr-11A_2 were respectively compatible with Pr-47A_2 and Pr-47A_1. Mating types were obtained by the isolation of single conidia from Pr-47 and Pr-11. Single ascospores from any source yielded only self-fertile clones. This behavior suggests that the wild-type self-fertile culture is diploid, although the site of meiosis is not yet known. Staining of vegetative and reproductive cells with Giemsa indicates that gametangia and mature ascospores each have a single nucleus, whereas arthrospores and vegetative hyphal cells have one to four nuclei. This condition suggests the possibility of somatic meiosis. The evolutionary tendency in the sexual strains appears to be toward self-fertility; nevertheless, self-sterile clones are most common in nature, and in this regard we have made several fertile crosses between field isolates of *G. candidum* from various parts of the world.

The perfect state of *G. candidum* is similar to *Endomyces reessii* van der Walt (*7*), whose asexual state may form part of the species complex of *G. candidum*. In this connection we have isolated self-sterile, cross-fertile mating types of *E. reessii*, and although morphologically similar to *G. candidum*, they did not form ascospores in any pairing with *G. candidum* (*8*).

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8. A Latin description validating the perfect state as *Endomyces candidus* is in preparation.
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Diploid Azaguanine-Resistant Mutants of Cultured Human Fibroblasts

Abstract. *Two azaguanine-resistant clones of cultured, human fibroblasts were isolated from unrelated strains of karyotypically normal, male cells. The most resistant mutant has little hypoxanthine-guanine phosphoribosyltransferase activity, is virtually unable to incorporate hypoxanthine (a normal substrate of the enzyme), and resembles fibroblasts cultured from boys with the Lesch-Nyhan syndrome. The less resistant mutant has about one-third as much enzyme activity as its parent strain and is less able to utilize hypoxanthine. Both mutants are morphologically and karyotypically normal. These mutations may have occurred at the X-chromosomal, hypoxanthine-guanine phosphoribosyltransferase locus and may provide a realistic experimental model for studying mutation in human genetic material.*

We report here the isolation of azaguanine-resistant mutant cells from cultures of karyotypically normal, male human fibroblasts. Biochemically related mutants have previously been derived from strains of human and mouse cells (*1*, *2*, for example), which were already hyperdiploid and prone to additional karyotype variation. Azaguanine-resistant mutants of essentially diploid Chinese hamster cells have been described (*3*), but we believe that the mutants described below are the first diploid, biochemically defined mutants of human cells to be isolated in vitro.

Selection for resistance to 8-azaguanine (AG) is based on the activity of hypoxanthine-guanine phosphoribosyltransferase [HG-PRT; E.C. 2.4.2.8 (*4*)], which is specified in humans by a gene on the X chromosome (*5*). Normal substrates for the enzyme are hypoxanthine and guanine, which are converted to inosine 5′-monophosphate (IMP) and guanosine 5′-monophos-