Where one host is present without the other, as in Greenland, arctic Alaska, and the southern Appalachians, neither rust has been reported. A possible exception is the Kaibab Plateau of northwestern Arizona, where *Peridermium coloradense* is abundant on spruce but bearberry has not been reported. It will be interesting to see whether another *Arctostaphylos* species serves as host, or the broom rust is short-cycling on spruce, or bearberry is actually present. *Peridermium coloradense* has been reported southward to central Mexico (*5*), far beyond the range of bearberry, but probably this is erroneous because central Mexico is also far south of the spruce host's range. In Eurasia both hosts are present, but both rusts are absent, according to mycological works on that area.

The bearberry *Chrysomyxa*, though called microcyclic (*2*), is on the "wrong" host to be so according to Transhel's law, which is (in part) that microcyclic rusts occur on the aecial hosts of related macrocyclic species (*6*). The aecial hosts of *Chrysomyxae* are *Picea* species, not Ericaceae. No evidence was ever presented that *Chrysomyxa arctostaphyli* is microcyclic; it was simply stated to be so. It provided the principal apparent exception to Transhel's generalization (*6*). Because the telia produced on bearberry from inoculation with *Peridermium coloradense* are identical with those of *Chrysomyxa arctostaphyli*, we can now assume that the latter is not a microcyclic species. Other possible exceptions are also species of *Chrysomyxa*, and are even less known than bearberry rust; quite likely they too are host-alternating. The "law" may apply to all microcyclic rusts.

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13 April 1961

Preference Factors in Experimental Alcoholism

Abstract. Normal rats which refused 5- and 20-percent alcohol in a previous study were restricted to 5-percent and 20-percent solutions in their home cages for either 30 or 120 days. Differential preferences for alcohol solutions of up to 8-percent were established as a function of length of time animals consumed alcohol but not as a function of the particular concentration consumed prior to testing.

One of the principal experimental procedures in physiological studies of alcohol consumption is the voluntary self-selection method. By daily increasing the percentage concentration of alcohol, Richter and Campbell (*1*) have shown that rats preferred alcohol over water in ranges of from 1.4 to 6.5 percent. Myers (*2*), however, found that rats which had never been exposed to alcohol refused a 5-percent solution and would not select this concentration in preference to water; this preference was reversed only when the rats were restricted to alcohol for at least 10 days. From this and other evidence (*3*), it seems that in Richter's experiments the gradual increases in concentration of alcohol modified the animals' preference threshold.

In order to clarify the role of the time and concentration factors in self-selection, 16 male, 300-day-old hooded rats of the Colgate strain were trained in boxes containing three levers (*4*) to obtain with each respective lever press a pellet of food, 0.03 ml of water, or 0.03 ml of 5-percent alcohol in one apparatus or 20-percent alcohol in the other (*5*). Each animal was deprived of food and alcohol for 24 hours, and during the 1-hour test session obtained its only food and fluid until the next day at the same time. In all cases the rats preferred water to both concentrations of alcohol throughout the 12 consecutive test sessions. Therefore, the rats were divided into four equal groups so that fluid intakes in their home cages were restricted to 5-percent alcohol for either 30 or 120 days, or 20-percent alcohol for 30 or 120 days. During this time they were maintained on their normal free-feeding laboratory food regimen. Retesting was then carried out, with half of the rats in each of the groups offered alcohol solutions which were increased from 5-percent, in 1-percent steps on sucessive daily test sessions, and the other half offered alcohol that was decreased from 15-percent concentrations in the same manner. As in previous research (*2*) the data showed that food responses (intake) were identical across all groups.

With respect to fluid preference as a function of the two alcohol concentrations in the home cages, there were no differences between the preference curves of rats that consumed 5-percent solutions and those that drank 20-percent solutions. The data from these two groups therefore were combined.

Figure 1 illustrates the preference functions based on the effects of increasing versus decreasing the order of alcohol concentrations offered during testing. Neither the 30- nor the 120-day group on the decreasing alcohol schedule manifested a clear-cut preference for alcohol until the concentration dropped to 4 percent (bottom graph). This is in sharp contrast to the preferences for higher alcohol concentrations by the groups offered alcohol increasing in concentration by 1 percent each day (top graph). Here it is postulated that the water preferred during the 9-day period in which the alcohol concentrations were high and in the aversive range reduced the acclimation to and counteracted the effects of the long-term drinking.

In Fig. 2 an over-all comparison of water and alcohol response functions, independent of the increasing or decreasing order, clearly shows that in the 30-day groups a shift in preference from alcohol to water occurs more

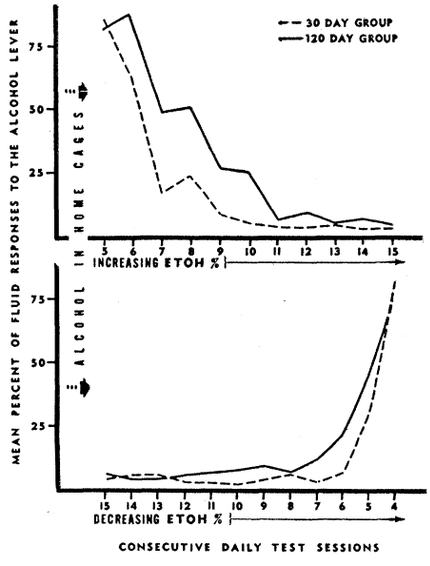

Fig. 1. Comparison of the two testing methods for offering alcohol solutions to both 30- and 120-day groups of rats. On successive days, the alcohol solution was increased by 1-percent steps (top) or decreased by 1-percent steps (bottom).

rapidly and at a significantly lower percent than in the 120-day groups ($P < .01$). Thus it may be concluded that although the home cage concentration of alcohol solution prior to testing did not seem to be significant in these tests, two important factors did influence the change in preference for alcohol: (i) the amount of time spent drinking prior to testing, and (ii) occurrence of an interval when water was ingested while alcohol was in the gustatorily noxious range.

Finally, in view of these and other data, it is apparent that the arbitrary selection of a predetermined alcohol solution, such as the commonly used 10-percent concentration (*6*), is a questionable procedure for studying those experimental variables affecting the preference of this substance. Attributing animals' refusal of 10-percent solution to some physiological condition or alteration may be entirely erroneous, since this concentration simply could be above the normal organism's maximum preference level (refer again to Figs. 1 and 2).

In investigations which utilize preference for alcohol (and probably other substances) as a main experimental effect, the following factors must be considered: (i) acclimation period or prior exposure to the substance; (ii) the preference threshold for the specific genetic strain of individual animals under investigation (*7*); (iii) the nutritional and metabolic states of the organisms (*8*); and (iv) technical details, including cage position of the available substance and possible stress conditions (*9*). Neglect of any or all of these factors could seriously affect the validity of the research (*10*).

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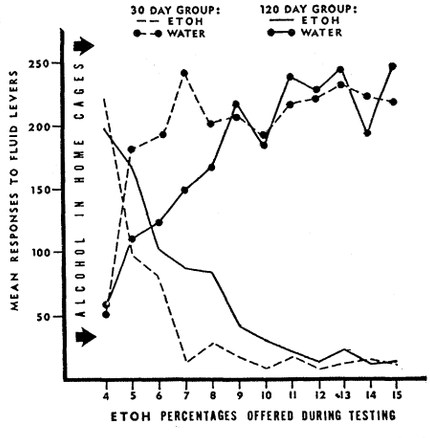

Fig. 2. Mean daily preferences for alcohol and water in rats whose fluid intakes in their home cages were restricted to 5-percent or 20-percent alcohol for 30 days and 120 days. These plots are based upon combined data of increasing-decreasing order groups and the 5- and 20-percent groups, and represent the mean fluid intakes on each 1-hour consecutive daily test session.

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Suppressor of Pyrimidine 3 Mutants of Neurospora and Its Relation to Arginine Synthesis

Abstract. The basis of the mutant phenotype of the *pyr 3a* strain of *Neurospora* appears to be the arginine sensitivity of an early step in pyrimidine synthesis. The effect of a suppressor mutation which renders *pyr 3a* pyrimidine-independent is to reduce arginine levels in the mycelium by its effect on ornithine transcarbamylase.

The *pyr 3* locus of *Neurospora* is represented by a number of independent mutations which impose a pyrimidine requirement upon the organism. It has been found that a group of mutants represented by *pyr 3d* (45502) lacks the enzyme aspartic transcarbamylase (ATC) (*1*) and does not respond to the presence in the same genome of an unlinked suppressor mutation, *s* (*2*). The mutants represented by *pyr 3a* (37301), on the other hand, display normal aspartic transcarbamylase activity (*1*), and the pyrimidine requirement is entirely or almost entirely eliminated in the presence of *s* (*2–4*). Previous work indicates that the *pyr 3a* mutation affects a step in the synthesis of pyrimidines which lies prior to the appearance of the product of the ATC reaction, ureidosuccinic acid (US) (*1*). Such steps may be the ATC reaction itself, or the availability of its substrates, carbamyl phosphate (CAP) or aspartic acid.

Direct attempts to identify the reaction affected in *pyr 3a* have been unrewarding. A study of the action of the suppressor, *s*, was undertaken, therefore, because it reverses the effect of the *pyr 3a* mutation. Very small concentrations of arginine (0.05 μg/ml medium) have been shown to restore a pyrimidine requirement to the suppressed mutant (*pyr 3a-s*) (*4*), indicating an inhibition of pyrimidine synthesis by arginine. Because ornithine transcarbamylase (OTC), catalyzing the formation of citrulline from carbamyl phosphate and ornithine, could well regulate arginine production, the effect of the suppressor gene upon this enzyme was investigated.

Growth conditions have been described previously (*1*). Acetone powder extracts were assayed for ornithine transcarbamylase activity by measuring the appearance of citrulline colorimetrically (*5*) in the following reaction mixture: 20 μmole of ornithine, 20 μmole of carbamyl phosphate, 250 μmole of tris acetate buffer, *p*H 9.0, and an aliquot of the extract; total volume, 3.25 ml; final *p*H, 8.7.

It was found that, under the conditions of the experiments, extracts of wild type, *pyr 3a* and *pyr 3d* mycelia at similar stages of growth displayed an ornithine transcarbamylase activity of 15 to 20 μmole of citrulline per milligram of protein per hour, while similar extracts of *pyr 3a-s* displayed activities of 0.2 to 0.6 μmole/mg per hour. Ascus analysis of a cross of *pyr 3a-s* to wild type showed that low OTC activity segregated regularly with suppressor action, and, where *s* was expected in otherwise wild type genomes, OTC activity was also low.

Strains carrying *s* (without *pyr 3a*) grew normally on minimal medium, despite the great reduction of OTC activity. Such strains were stimulated, if at all, by only 6 percent in linear growth rate when arginine was added to the medium, while all wild type strains were unaffected by the addition of arginine. This finding indicates that growth was limited only slightly by the lowered OTC activity, and that the inhibition of *pyr 3a-s* by arginine is a function of the *pyr 3a* mutation rather than of *s*. Most important, the data strongly suggest that the basis of the