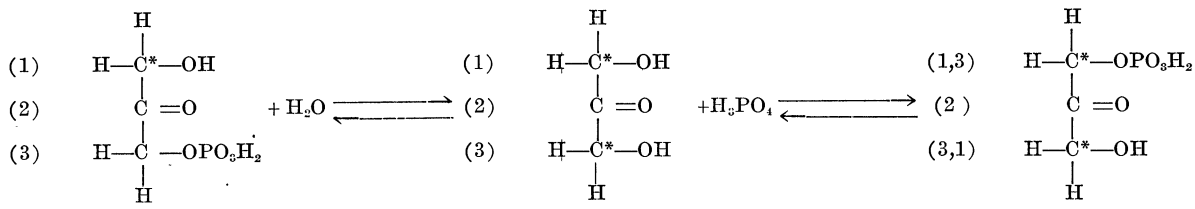

FIG. 2.

alkaline rearrangements resulting in lactic acid (*5*) the carboxyls may stem from carbons one and six. This is certainly so in the case of gluconic acid, where 1.3 moles of lactic acid per mole of gluconic acid can be obtained.

These experiments are in general accord with the recent note by Koshland and Westheimer (*3*) on the fermentation of glucose-1-C^{14} by yeast. In yeast fermentation, the carbon dioxide may be derived from a variety of intermediary products of metabolism. Furthermore, the ability of yeast to fix carbon dioxide is pronounced. In view of these and the rather large counting deviations in their experiments, a quantitative interpretation of their results is not possible.

At least three apparent explanations of our results are suggested, the latter two of which represent deviations from the normal fermentation mechanism: (a) that metabolic carbon dioxide (from the radioglucose) is reversibly reincorporated in lactic acid, in which case the activity would reside primarily in the carboxyl group, (b) that there is a fermentation mechanism in which carbons one and six rather than three and four are oxidized, (c) during the course of the normal fermentation mechanism the triose phosphates are in (partial) equilibrium with the free trioses, as in Fig. 2. This would, of course, result in activity appearing in both carbons one and three of lactic acid, to an extent depending on the amount of approach to an equilibrium. We have not investigated the enzyme systems involved and are thus not able to choose between the second and third explanations. The first explanation appears difficult to reconcile with the fact that the fermentation is conducted in M/10 bicarbonate (pH 6.8), with subsequent dilution of carbon dioxide formed by metabolism. Furthermore, control experiments with inactive glucose but with radiocarbonate (2.8×10^6 disintegrations per min in 5 ml of reaction mixture) resulted in approximately 0.02% of the activity in the lactic acid formed. There still remains the possibility, albeit dubious, that the metabolic carbon dioxide is preferentially used at the site of formation, prior to the diffusion and subsequent dilution effects of the solution's inactive bicarbonate.

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Diurnal Responsiveness of *Erysiphe graminis* to Nutrients

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Because it can be grown readily on barley in the greenhouse throughout the year, because the conidia germinate well on various culture media, and because germ tube growth is sharply increased by a number of nutrient chemicals, the fungus *Erysiphe graminis* D.C. appears well suited to studies of growth *in vitro* of a powdery mildew. No member of the Erysiphaceae has been cultured on a nonliving substrate, and all are commonly regarded as obligate parasites.

Previous studies of *Erysiphe graminis* have indicated no diurnal cycle in the germinability of the conidia or in the division of the generative cell such as is exhibited by *Erysiphe polygoni* (*1*). On an agar substrate, germ tube growth of *E. graminis* is increased by crude animal and plant extracts, especially a solution of hen's egg yolk; by iron sulfate, sucrose (*2*), disodium α-tocopherol phosphate, and Tween 60 (*4*). More recently, a medium consisting of 0.8% agar, 0.6% fructose, and 0.003% animal lecithin has been used. Using this medium, there has been great variation in germ tube length in different trials; such variation being associated, apparently, with the time of day the trials were started.

To check on the effect of time, plates of plain agar and lecithin-fructose agar, prepared simultaneously and stored at 0° C, were seeded with conidia of *E. graminis* from greenhouse plants at various times throughout the day and incubated at 16° C in the dark, and the germ tube growth was measured 5 days after seeding. Results of the first trial, started on February 11, 1949, are presented in Fig. 1, and indicate that germ tube growth on nutrient agar was greatest from seedings made about 12 M., and poorest from seedings made at 8:30 A.M. The germ tubes of the 12:30 P.M. seeding were 85 μ greater or 175% greater than the germ tubes of the 8:30 A.M. seeding. Germ

tube growth on nutrient agar was 40% greater than growth on plain agar at 8:30 A.M., and 236% greater

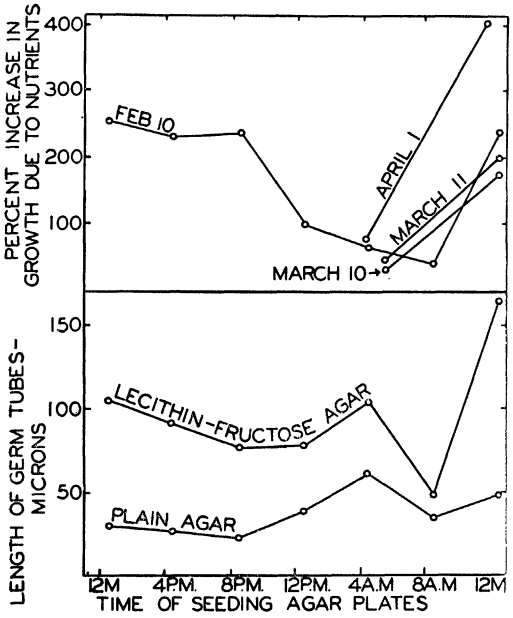

FIG. 1. Diurnal cycle of responsiveness of conidia of *Erysiphe graminis* to lecithin-fructose agar. Each value is the average of 20 germ tubes. Data of the 2 lower graphs are for February 10–11, 1949. The weather was cloudy before and during these tests and also during those of March 10 and 11. The weather was clear before and during the test of April 1.

than on plain agar at 12:30 P.M. In three subsequent trials, an attempt was made to compare only the approximate peak and trough of this diurnal cycle of responsiveness to nutrients. In these trials, the increase in germ tube length due to nutrients averaged 56% for seedings made from 4:15 to 5:30 A.M., and 244% for seedings made from 11:50 A.M. to 12:20 P.M. (see Fig. 1). In all trials, germination ranged between 25% and 92% and showed no apparent diurnal cycle and no apparent correlation with germ tube length.

Concurrent attempts to study in a similar way germ tube growth *in vitro* of *Erysiphe polygoni* from bean, and *Uncinula necator* from grape (both fungi show a clear diurnal cycle of conidiophore maturation, and the bean fungus also shows diurnal variation in sensitivity to copper sulfate [*3*]) failed to evince any marked response to the lecithin-fructose medium at any time tested. This indicates that there are specific differences between closely related species of powdery mildews with respect to nutrient response.

As the principal index of the nutrient value of a test chemical is the difference in growth of germ tubes on media with and without the test chemical, this diurnal responsiveness to nutrients, as in the germinability of the conidia of *E. polygoni* (*1*), is apparently the result of diurnal changes in the fungus while grown on the host plant before use in these tests. Diurnal responsiveness may be of considerable importance in studies of the growth *in vitro* of some powdery mildews.

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A New Method for the Study *in Vitro* of Rumen Digestion

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The utilization of cellulose has long been recognized as a primary factor in the nutrition of ruminants and particularly so among those subsisting largely on roughages. It is believed, therefore, that the rate of cellulose digestion *in vitro* by the microorganisms of the rumen may be used as a measure of how nearly it is possible to approach cellulose digestion *in vivo*.

A technique for the study of digestion in the rumen *in vitro* has recently been published by Marston (*3*). His procedure appears to simulate more closely the natural environment in the rumen than any earlier investigations. There is, however, one significant omission. No provision is made for the removal of nongaseous fermentation products which, as they accumulate, might be expected to slow the rate and eventually inhibit digestion.

To test the influence of such an accumulation of breakdown products, cellulose digestion was compared after incubation with fresh rumen fluid in a closed vessel (the technique of Marston), and in a semipermeable bag suspended in a large volume of aqueous growth medium.

Samples of rumen liquor were collected by scooping semisolid material from the lower regions of the rumen of a fistulated cow and straining this through three layers of cheesecloth, pressing the residue by hand directly into a glass container in which the air had previously been replaced by nitrogen or carbon dioxide. To minimize heat losses, the receptacle was wrapped in paper. After thorough mixing, 700-ml aliquots of this liquor were incubated with 12.0 (and later 20.0) g of Whatman #12 filter paper (ground in a Wiley mill to pass the 0.5 mm sieve), 15.0 ml of 1 M KH_2PO_4, and 7.5 ml each of 2 M $(NH_4)_2SO_4$, 1 M $MgSO_4$ and 0.5 M $CaCl_2$ in a glass bottle and in a semipermeable bag, using the type of apparatus presented in Fig. 1. Two fermentations were conducted at one time in duplicate apparatus, differing only in vessel *B*, which in one was a glass bottle, and in the other a "Visking" cellulose sausage casing of 4½ in. diam. In both, vessel *B* is closed by *a*, which is a wood stopper surrounded by a rubber gasket and coated with wax, and suspended in a 12 × 12-in. water bath *H*. Tubes *c* and *d* serve as inlets and outlets for gas, respectively; tube *e* is connected to a burette containing 0.5 M Na_2CO_3; *g* is a mercury-sealed shaft of an adjustable speed stirrer;

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