crease in size of the slower moving components, B, paralleled the increase previously shown to occur when pullets begin egg production (*6*). In the one case attempted, the same serum protein changes were observed in a 2-year-old male bird that had been treated with the hormone.

These results demonstrate that the injection of diethylstilbestrol will cause changes associated with egg formation to occur in the serum proteins of male birds. As previously mentioned, similar changes have been noted in the total calcium and phosphorus and in the calcium and phosphorus partition of pigeon serums. From these results it can be concluded that the increase in the serum proteins, which binds the increased calcium in the laying hens so that the total diffusible calcium remains constant, may be caused by the female sex hormone. The properties of the protein fractions found in the serums of normal and estrogen-treated male birds are under investigation.

References

1. RIDDLE, O., and REINHART, W. H. *Am. J. Physiol.*, **76**, 660 (1926).
2. HUGHES, J. S., TITUS, R. W., and SMITS, B. L. *Science*, **65**, 264 (1927).
3. HELLER, V. G., PAUL H., and THOMPSON, R. B. *J. Biol. Chem.*, **106**, 357 (1934).
4. RIDDLE, O., and MCDONALD, M. R. *Endocrinology*, **36**, 48 (1945).
5. MCDONALD, M. R., and RIDDLE, O. *J. Biol. Chem.*, **159**, 445 (1945).
6. BRANDT, L. W., CLEGG, R. E., and ANDREWS, A. C. *Ibid.*, **191**, 105 (1951).

Carbon Dioxide and Root Hair Development in *Anacharis* (*Elodea*)[1]

Hugh M. Dale[2]

Department of Botany,
University of Toronto, Toronto, Canada

Considerable interest has been shown in the fact that the normally hairless roots of *Anacharis* become coated with root hairs when they penetrate the mud. In a recent paper (*1*) two attempts at explanation are reviewed. In the first, soil particles are regarded as a stimulus and, in the second, light is considered the deciding factor. Cormack (*2*) and King (*3*), by completely excluding all light, reported abundant production of root hairs in water alone.

In an effort to understand the effect of light on the root, Cormack made a microscopic study. Unlike the roots grown in light, those grown in darkness had no cuticle. He assumed that the toughness of the cuticle prevented the extension of the epidermal cells as root hairs. Roots grown in the light had chloroplastids in the epidermal cells, whereas these were absent in roots grown in the dark. Lee and Priestley (*4*) had pointed out that the saturation of fats by oxygen is one stage in the formation of cuticle. Unsaturated fatty materials migrating to the surface in the presence of oxygen would be toughened into cuticle. Cormack considered the oxygen produced in the epidermal cells of the root during photosynthesis to be involved in this reaction. In the absence of light no oxygen would be produced, and the unsaturated fats would wash away without being changed. He tested this by administering ethylene gas to inhibit chlorophyll formation. After this treatment the usual stains gave no evidence of a fatty layer, and root hairs were produced in the light.

While investigating the development at the stem apex, the writer has frequently grown the cultivated and native *Anacharis* in nutrient solutions of high carbon dioxide tension. A continuous stream of minute bubbles of this gas flowed through the solution in covered 4-liter Pyrex jars. Under this treatment the green roots of *Anacharis* grown in light were invariably covered with root hairs. There was an abundant supply of oxygen, for as soon as the carbon dioxide tension built up the sprigs were buoyed up by the increased oxygen in the air spaces, and it was necessary to weight the plant down by pierced sections of glass slide to keep it immersed. Presumably carbon dioxide concentration is often the limiting factor in photosynthesis. On staining with Sudan IV, a fatty layer on the outside of the roots was observed, the root hairs breaking through this layer. This would seem to contradict Cormack's evidence; nevertheless, a study of the nature of this fatty layer was undertaken. On warming sections of the roots in 10% potassium hydroxide, the hairless root lost only a small part of its staining film, whereas the root grown in high carbon dioxide tension completely lost its coating of fatty material, which therefore could not be considered as cuticle. To discover if the fats were oxidized, a 1% aqueous solution of osmic acid was used. It is reduced and blackened if the fats are unsaturated. Soaking sections of hairless roots in the solution overnight produced a slight darkening on the outside of the fatty layer. Only a very thin outer layer was still unsaturated. The hairy roots produced in abundant carbon dioxide showed a definite blackening in the whole thickness of the layer. After saponification with the alkali no darkening of the epidermal walls occurred in either hairy or hairless roots, although the cuticle was still retained on the latter. The carbon dioxide in some way had prevented the oxidation of fats to form a cuticle, and the formation of root hairs was not prevented.

Another problem presented by the high carbon dioxide tension is that of pH. Cormack (*5*) in his investigations on *Brassica* has shown that the formation of root hairs is linked with the change of pectic acid to calcium pectate in the outer wall of the epidermis. He demonstrated that this took place in cells having a pH above 5.8, none developing on cells of a pH of 4.6–4.8. In the writer's experiments the pH of the solution in which the plants were grown was 3.8. In order to understand this discrepancy, a crude attempt

[1] The investigation was made possible through the encouragement and interest of H. B. Sifton and the financial assistance of the Department Veterans Affairs, Canada.

[2] Present address: St. Andrews College, Aurora, Ontario, Canada.

was made to measure the pH very close to the plant. The plants were removed from their jars and given a quick shake, and the film of water on their surface was centrifuged off. The pH was found to be 0.5–1.0 above that of the surrounding medium and of the whole crushed plant. The pH of the epidermal cells must presumably be even higher, as the cell walls contain calcium pectate. Ruthenium red gave the characteristic stain even after heating sections for one hour in 2% ammonia at 90° C, which would remove any pectic acid or pectin.

References

1. CORMACK, R. G. H. *Botan. Rev.*, **15**, 583 (1949).
2. ———. *New Phytologist*, **36**, 19 (1937).
3. KING, L. J. *Botan. Gaz.*, **105**, 127 (1943).
4. LEE, B., and PRIESTLY, J. H. *Ann. Botany*, **38**, 525 (1924).
5. CORMACK, R. G. H. *New Phytologist*, **34**, 30 (1933).

The Inhibitory Effects of Sorbose on Fungi[1]

H. L. Barnett and V. G. Lilly

Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown

The fungi vary greatly in their ability to utilize different sugars as sources of carbon. Few fungi have been reported as being unable to utilize glucose or maltose (*1–3*), both of which are common, naturally occurring sugars. The utilization by certain fungi of other common sugars has been studied extensively (*4–9*). On the other hand, little is known of the utilization of the so-called rare sugars and of those that are not common in nature. Sorbose is one of the latter. It is formed from sorbitol, by the action of *Acetobacter suboxydans* (*10*). When present in a medium as the sole source of carbon, sorbose is utilized well by some fungi but only poorly or not at all by other species.

An unusual inhibitory effect of sorbose on the utilization of an available sugar, such as glucose, maltose, or sucrose, was discovered when certain fungi were grown in media containing a combination of sugars. The results of these studies are presented below.

The basal medium used was composed of asparagine 2 g, KH_2PO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, microelements as sulfates, Fe 0.2 mg, Mn 0.1 mg, Zn 0.2 mg, thiamine 100 µg, biotin 5 µg, and double-distilled water, 1,000 ml. Unless otherwise stated the amount of each sugar was 20 g/l. The media were adjusted to a pH of 6.0 (except in the study of the effects of pH), dispensed in 25-ml lots into 250-ml Erlenmeyer flasks, and autoclaved at 15 psi for 15 min.

The media were then inoculated, using uniform amounts of actively growing mycelium on agar, and thus eliminating any possible effects resulting from restricted or delayed spore germination. The cultures were incubated in a constant-temperature room at 25° C, except in temperature experiments, when refrigerator-incubators and water-jacketed incubators were used. Replicates of 4–10 cultures were used, and 2–6 cultures were harvested at the same time. Harvests of mycelium were accomplished by filtering the excess liquid through a fine cloth, drying the mycelial mats at 90° C for 12 hr, and weighing.

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 442.

TABLE 1

GROWTH OF 10 FUNGI IN 25-ML LIQUID MEDIA CONTAINING SINGLE SUGARS OR MIXTURES OF SUGARS
(Av dry wt of 2–4 cultures grown at 25° C)

Fungus	Days	Glucose	Glucose-sorbose	Maltose	Maltose-sorbose	Sorbose	Glucose (40 g/l)
Ceratostomella fimbriata	5	113	T*	84	T*	T*	107
Chaetomium globosum	12	373	292	364	25	T*	375
Alternaria solani	8	99	81	140	33	20	196
Sphaeropsis malorum	5	153	140	166	61	T*	240
Endothia parasitica	9	201	281	191	162	11	167
Choaneophora cucurbitarum	5	75	104	44	46	T*	100
Polyporus versicolor	9	152	232	126	192	47	221
Aspergillus rugulosus	13	239	439	200	221	189	448
Botrytis cinerea	9	182	266	210	310	198	379
Fusarium tracheiphilum	4	209	218	205	247	183	264

* T = trace of growth, estimated as less than 10 mg.

When a number of fungi were grown on sorbose, alone or in the presence of other sugars, it was noted that the response varied greatly. In general, the fungi fell into three groups: (1) growth on maltose greatly inhibited by the presence of sorbose, with little or no growth on sorbose alone; (2) growth on maltose-sorbose medium approximately the same as on maltose alone, but growth quite poor on sorbose alone; (3) fair to good growth on sorbose, as compared to maltose and maltose-sorbose media. The growth of representative fungi on different sugars is shown in Table 1.

C. fimbriata showed the greatest inhibition by sorbose. Under no condition did the cultures make more than 10–15 mg of mycelium in media containing 20 g sorbose/l, even in the presence of glucose or maltose. Additional experiments not reported here showed that smaller amounts of sorbose in the presence of glucose or maltose caused less inhibition. It is apparent that the presence of sorbose in the medium actually interferes with the absorption or the utilization of glucose and maltose by some fungi. It is also evident from Table 1 that the inhibition due to sorbose is greater in combination with maltose than with glucose.

The effect of temperature was then determined by culturing fungi previously shown to be inhibited by sorbose at temperatures ranging from 15° to 35° C.