These brass rings are 1 mm thick with a 25-mm outside diam and a 19–20 mm inside diam. About one-fourth of the ring is cut away, leaving it incomplete. The ring, held by forceps, is heated over a flame and then dropped into a dish of salvoline (6 parts vaseline and 1 part paraffin). It quickly sinks below the surface. It is then picked up, the excess salvoline is drained off and the ring, now completely covered with salvoline, is placed on an ordinary glass slide, well cleaned with 70% alcohol. When cool and hard, the salvoline holds the ring in place. The space enclosed by the ring is then filled with distilled water, most of which is at once drained off, leaving the surface of the slide within the ring covered with a thin film of water.

The cover glass containing the tissue culture is removed from its depression slide and placed on the brass ring. The salvoline holds it firmly in place. The layer of water inside the ring keeps the atmosphere around the culture saturated with water at a fairly constant temperature, so that water does not condense on the cover slip. If necessary, more water can be added to the space in the ring by means of a fine pipette placed at the opening in the ring. The tissue culture never comes in contact with the water. After photographing, the cover slip with its tissue culture is returned to its glass depression slide and the brass ring is ready for use again. Sometimes it may be necessary to add more salvoline to the top of the ring. This is easily done with a glass pipette filled with salvoline. The slide inside the ring must be completely free of salvoline. If it is not, then a freshly cleaned slide must be used; otherwise the distilled water will not form a thin even film over the glass slide. Glass rings or plastic rings can be used instead of brass rings.

Enzymatic Decomposition of Lignin[1]

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Numerous claims have been made in the literature purporting to demonstrate the ability of microorganisms to utilize lignin as a sole carbon source, or purporting to demonstrate the existence of enzymes acting specifically on the lignin molecule. These reports are based on techniques which presuppose that lignin isolated by acid or alkaline treatment of plant material undergoes no important changes during its isolation (*8, 9, 10, 13, 15*) or that various color reactions can be used to detect the presence or disappearance of lignin (*2, 6*). Some work has been based on the assumed similarity in structure between lignin and tannic acid (*1*). Advances in our understanding of the chemistry of lignin indicate that these suppositions are very dubious (*5, 12, 14*). The work of Brauns (*3, 4*) in isolating so-called "native lignin" by a process which minimizes structural alterations in the lignin molecule seemed to us to warrant an experimental reexamination of the ability of microorganisms to utilize isolated lignin and of the possible existence of lignin-oxidizing enzymes. Results of these studies to date are summarized here in a preliminary manner.

Lignin was prepared from 12-yr-old red sprucewood by the method of Brauns, and used in both nutritional and enzyme studies with fungi.

After a survey of a large number of species of wood-rotting fungi, good growth of several of these organisms was obtained after two weeks' incubation on media in which lignin was the limiting carbon source. This material will be reported in a separate communication (*7*).

Studies on a lignin-oxidizing enzyme were carried out, using the absorption of oxygen measured in a Warburg manometric apparatus as an indication of enzyme activity. A reliable source of enzyme was found to be commercial mushroom spawn,[2] which is an intimate mixture of mycelium of *Agaricus campestris* and well-decomposed horse manure. Conditions used in the preparation of this material are such that it is essentially a pure culture of this organism. A dry, stable preparation of the enzyme has been obtained by the following procedure:

Mushroom spawn is mixed with three times its weight of distilled water in a Waring Blendor for 3 min. The resulting mash is subjected to a pressure of 15,000 psi in a Carver press and the press juice is dialyzed for 48 hr in Visking cellulose sausage casing against cold running tap water. The dialyzed solution is removed from the casing, cooled to 5° C, and to it is added two volumes of cold acetone. The resulting precipitated solution is cooled to 5° C, and the brown precipitate is centrifuged down and washed twice with cold 66% acetone. The precipitate is finally dried at 35° C under aspirator pressure. One lb of spawn yields 2–3 g of this dry preparation. The concentration of solids is reduced from 20 mg/ml in the original press juice to 6 mg/ml in the dialyzed solution, and to 2 mg in the acetone precipitate.

The enzyme is activated by citrate and phosphate ions and it is therefore buffered to pH 6.0 with McIlvaine's buffer (*11*) for activity measurements. Under these conditions 75 cu mm of oxygen are absorbed in the first hour when 1 ml of an aqueous solution containing 3 mg of enzyme is incubated with 1 ml of an aqueous lignin suspension containing 30 mg lignin/ml. The enzyme is quite stable in acid solution (pH 4.0–6.5), but loses activity rapidly at pH values higher than 7. The pH optimum for enzyme activity is 5.7–6.0, with a temperature optimum close to 40° C. Under optimum conditions, the activity of a solution of enzyme is proportional to its concentration.

It is believed that this enzyme is not identical with any of the known phenol oxidases, since mushroom sporo-

[1] This work was done under Contract No. N7 onr 397-4 between the University of Maryland and the Office of Naval Research. The project was initiated at the suggestion of the Prevention of Deterioration Center, National Research Council.

[2] The mushroom spawn used in these experiments was obtained from the L. F. Lambert Company, Coatesville, Pennsylvania.

phores (a recognized source of tyrosinase) do not yield a product having activity. The enzyme is not activated by copper ions and is completely inactivated at pH values higher than 7. A study of the action of various enzyme inhibitors supports the nonidentity of this enzyme with any of the known copper-containing enzymes.

Studies are now in progress on the effect of this enzymatic reaction on the structure of the lignin molecule and on further purification of the enzyme. A detailed account of the work summarized here will be published in another journal.

References

1. Bavendamm, W. *Ber. Deut. Bot. Ges.*, 1927, **45**, 357.
2. Bayliss, J. S. *J. econ. Biol.*, 1908, **3**, 1.
3. Brauns, F. E. *J. Amer. chem. Soc.*, 1939, **61**, 2120.
4. ———. *J. org. Chem.*, 1945, **10**, 211.
5. Crocker, E. C. *Ind. eng. Chem.*, 1921, **13**, 625.
6. Czapek, F. *Ber. Deut. Bot. Ges.*, 1899, **17**, 166.
7. Day, W. C., Pelczar, M. J., and Gottlieb, S. *Arch. Biochem.* In press.
8. Fernandez, O. and Regueiro, B. *Farm. nueva*, 1946, **11**, 57.
9. Garren, K. H. *Phytopathology*, 1938, **28**, 875.
10. Kazanskii, A. S. and Mikhailova, M. A. *Lesokhim. Prom.*, 1936, **5**, 16.
11. McIlvaine, T. C. *J. biol. Chem.*, 1921, **49**, 183.
12. Norman, A. G. *Science Progress*, 1936, **30**, 442.
13. Waksman, S. A. and Hutchings, I. J. *Soil Science*, 1936, **42**, 119.
14. Wise, L. E. *Wood chemistry*. New York: Reinhold, 1944. Pp. 272–369.
15. Zobell, C. E. and Stadler, J. *Arch. Hydrobiol.*, 1940, **37**, 163.

The Hemostatic Activity of Amniotic Fluid[1]

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During studies of the coagulation mechanism in pregnancy it seemed of interest to investigate the action of amniotic fluid. This fluid was collected in a sterile syringe by aspirating the intact membranes of women in labor. The Lee and White clotting test was done, using four 12 × 75-mm glass tubes. One-tenth cc of amniotic fluid was added to each of two tubes and an equal amount of saline to each of two control tubes. Freshly drawn venous blood was added in 2-cc amounts to all four tubes, which were then incubated at 37° C. The end point taken was that at which both tubes of each set could be inverted completely without spilling. Results are shown in Table 1.

It can be seen that one part amniotic fluid, when added to twenty parts of blood, can cut in half the time required for clotting.

It appeared that by studying the behavior of amniotic fluid with oxalated plasma and fibrinogen solution[2] we might be able to identify its place in the coagulation mechanism. The effect of amniotic fluid on the rapidity of clot formation in recalcified plasma is illustrated in Table 2. These data indicate that amniotic fluid decreases the clotting time of recalcified oxalated plasma.

TABLE 1

Clotting Time

Specimen	Number of tests	Controls	With amniotic fluid
		Min	*Min*
A1-19	5	7.0	3.5
A1-21	3	7.5	3.0
A1-20K	4	7.8	3.4
A1-19W	3	7.7	3.4
A1-25S	6	7.5	6.0
A1-27	3	5.8	4.2
A1-28	7	6.3	3.6
A1-30G	15	8.0	3.6
A1-31	4	8.7	2.8
A2- 4	7	10.0	3.9
A2- 4S	3	8.0	2.6
A2- 5A	8	9.0	4.0
A2- 5N	5	9.3	2.7
A2- 5S	5	9.6	3.4
A2- 9W	5	7.5	3.6
A2-15A	4	8.3	3.5
A2-24	4	7.7	3.1
A2-25B	8	10.0	3.7
A2-24N	3	7.5	3.1
A3- 1G	5	8.7	2.4
A3- 2W	5	9.2	3.9

TABLE 2

Recalcification Time

Specimen	Controls*	Plus 0.1 cc amniotic fluid†
	Sec	*Sec*
A1-19	76	46
A1-21	85	55
A1-20K	100	60
A1-19W	85	25
A1-25S	142	71
A1-27	131	55
A1-28	177	52
A1-30G	145	42
A1-31	111	47
A2- 4	140	56
A2- 4S	125	49
A2- 5A	93	53
A2- 5N	97	37
A2- 5S	110	61
A2- 9W	93	56
A2-15A	115	59
A2-24	135	65
A2-25B	145	73
A2-24N	145	63
A3- 1G	137	61
A3- 2W	120	36

* Recalcification time for 0.1 cc of 0.025 *m* calcium chloride, plus 0.1 cc physiologic saline, and 0.1 cc fresh plasma (0.5 cc of 0.1 *m* sodium oxalate to 4.5 cc blood).

† Substituting 0.1 cc fresh amniotic fluid for saline in recalcification.

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[2] Supplied by courtesy of Dr. John Edsall, Department of Physical Chemistry, Harvard Medical School, Boston.