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The Precipitin-Ring Test Applied to Fungi.*

GEORGE K. K. LINK, ADELINE DE S. LINK, GEORGE L. CROSS AND HAZEL W. WILCOX. (Introduced by F. C. Koch.)

From the Departments of Botany and Chemistry, University of Chicago.

During a study of the phenomena of resistance and susceptibility of plants to pathic events, experiments have been made to determine whether serological techniques could be used as tools for the study of phytopathogenic microparasites, and of their parasitic and pathogenic relations to plants. In the course of experiments from 1925 to 1928 it was found that in many cases serological specificity of phytopathogenic schizomycetes as manifested in the agglutination test apparently is correlated with host, and even symptom specificity within the same host. Incidentally, Sharp made the first report of finding a smooth virulent, and a rough avirulent strain of a phytopathogenic bacterium, *Phytomonas phaseoli sojense,* which parasitizes the soy bean plant.[1]

We chose next to determine whether serological techniques could be used in the study of phytopathogenic fungi and their nonpathogenic taxonomic allies.† To put the methods to severe test we chose to begin with members of the form genus *Fusarium,* phytopathogenically the most important genus of the division *Phragmosporae* of the so-called "fungi imperfecti". According to Wollenweber[2] this genus includes 64 species, with 79 varieties and 38 forms. These have been arranged in 16 sections with 9 subsections, 12 of

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[1] For a summary and literature citations of most of this work and of relevant work by others with phytopathogenic bacteria, see Link, G. K. K., Chap. 44, p. 590-606, in Jordan, E. O., and Falk, I. S., "The Newer Knowledge of Bacteriology and Immunology," University of Chicago Press, Chicago, 1928. 1196.

† This work was begun by 2 of us (G. K. K. L. and A. DeS. L.) in 1927 with equipment provided us in the Department of Hygiene and Bacteriology through the courtesy of Dr. W. H. Taliaferro. In 1929 a grant was received from the Rockefeller Foundation, and since then the work has been carried on uninterruptedly with the assistance of Dr. George L. Cross (1929-1930) and of Hazel W. Wilcox (1930- —). Upon completion of the New Botany Laboratory, made possible in part by a grant to the University from the General Education Board, the work has been carried on in the Botanical Laboratories.

[2] Wollenweber, H. W., *Fusarium Monographie, Fungi parasitici et saprophytici. Z. für Parasitenkunde. Abt. F. Zeitschrift für wissenschaft. Biol.,* 1931, **3**, 19.

the sections including members for which the perfect ascomycete stage is known. This genus includes many parasites which are part of the etiological complex of interesting and economically important plant diseases. Identification and classification of the Fusaria is often difficult or impossible because when grown on artificial media they may fail to produce the characteristic structures necessary for their differentiation.

The work was begun with 2 species, *F. conglutinans,* incitant of cabbage yellows, and *F. cubense,* incitant of banana wilt. In the course of the past 5 years, as different problems of technique arose, 19 species of Fusarium have been used as inject and/or as test antigens against all the others. These are: *F. anthophilum, F. argillaceum, F. conglutinans, F. cepae, F. cubense, F. decemcellulare, F. dimerum, F. fructigenum, F. graminearum (Gibberella Saubenetii), F. javanicum, F. lycopersici, F. moniliforme, F. nivale, F. ossiculum, F. oxysporum, F. semitectum, F. sporotrichioides, F. theobromae,* and *F. trichothecioides.* In addition *F. conglutinans var. callestephi,* 2 strains of *F. cubense,* and one strain of *G. Saubenetii* have been used. *F. acuminatum, F. sambucinum,* one species each of Ramularia and of Cylindrocarpon, *C. album,* one strain of *Sclerotinia sclerotiorum,* and the + and— strains, singly and jointly of *Neurospora tetrasperma* have been used as inject and/or as test antigens, against the antiserum of one or more of the other inject antigens.

Since no serological studies of these organisms were reported until our work was in progress[3] considerable preliminary experimentation had to be done. The following problems have been investigated:

1. Search for a suitable protein-free medium revealed that Richard's solution‡ could be used for all the fungi tested. Good mats usually developed in subdued light at 20-30°C. in 3-4 weeks.

2. A method has been developed for recovering the fungus mats from these cultures, drying them with a minimum of autolysis, and rapidly preparing fungus powders which pass a 100 mesh sieve.

3. Studies were made to determine the best treatment of powders to obtain saline extracts with maximum antigen content.

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[3] Coons, G. H., and Strong, M. C., Mich. Acad. of Sciences, Arts and Letters, 1929, **9**, 65.

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	gm.		gm.
Sucrose	33.3	KH_2PO_4	3.3
KNO_3	6.6	$MgSO_4$	1.7
	H_2O (distilled)	1000.0 cc.	

4. Experiments were made to determine the relative merits of agglutination, precipitin, and precipitin-ring tests. The last was found to be the most feasible.

5. Micro-Kjeldahl analyses were used as a basis for calculation of the amounts of protein in each powder and saline extract in an attempt to correlate the amount of protein in the inject and test antigens and the titres obtained in the serological tests.

6. The relative merits of intravenous and/or intraperitoneal injection of rabbits were determined. Intravenous injection of the clear saline extracts of the powders and intraperitoneal injection of saline re-suspensions of the extracted residues were found best.

7. For the production of most specific titres the optimal dosages, intervals of injection, and bleeding after sensitization were determined.

8. The effect upon the quality and titre of the antisera of age, moulting, and temperature of environment of the rabbits, as well as the effect of temperature and time upon the antisera *in vitro* were determined.

9. Studies were made to determine the best method of preparing test antigens suitable for the precipitin-ring test. Grinding of the powders with ligroin-extracted pumice and their subsequent extraction with petrol ether (ligroin, B.P. 30-60°C. Max. B. P. 80°) preliminary to extraction at 0°C. with saline for 18 hours, followed by centrifugation and filtration through coarse filter paper, were found adequate to yield clear, potent test-antigens.

10. Studies were made of the layered tubes with respect to the incubation temperature and reading intervals most conducive to detection of clear rings and maximum titres.

11. Tests were made to determine the relative efficacy of undiluted antisera, and of antisera diluted with glycerine, glucose, and hypertonic salt solution, when tested against progressive dilutions of the test antigens. The undiluted antisera were most satisfactory.

12. Test antigens were prepared with buffered saline solutions to determine the effect of pH upon the range and specificity of the reactions.

13. Absorption tests were made to differentiate between closely related antigens.

14. A study of skin tests has been begun to further investigate correlation of serological and morphological relationship of the fungi.

All fungi tested are antigenic as determined by the precipitin-ring test. Titres up to 1:25,600 have been obtained.

All but one of the animals tested (120) produced antisera. The normal sera of very few (2) gave feeble reactions above the lower dilutions (1:200) of the test antigens.§

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Influence of Acute Infection and of Artificial Fever on Plasma Lipoids.

IRVINE MCQUARRIE AND A. V. STOESSER.

From the Department of Pediatrics, University of Minnesota Medical School.

During another investigation on the lipoids of the plasma in children, it was observed in several instances that values for lecithin, cholesterol and total fatty acids were all markedly influenced by acute infections. Although the literature revealed the fact that cholesterol has previously been found to be lowered in certain acute infectious diseases[1, 2] and in tuberculosis,[3, 4] little is actually known

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1 Denis, W., *J. Biol. Chem.*, 1917, **29**, 93.

2 Kipp, H. A., *J. Biol. Chem.*, 1920, **44**, 215.

3 Eichelberger, L., and McCluskey, K. L., *Arch. Int. Med.*, 1927, **40**, 831.

4 Henning, B. H., *J. Biol. Chem.*, 1922, **54**, 167.