laender in that the axial fibrils of ram spermatozoa are continuous from the head through the neck to the midpiece. In addition, the micrographs show clearly that there are 9 fibrils up to the head-neck junction in ram spermatozoa. The axial filaments that constitute these fibrils and the microstructure of spermatozoa from other species are under investigation. Detailed results will be reported later.

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A Fungistatic Substance Extracted from Vitrain

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In 1951 W. D. Evans (*1, 2*) reported the presence of a bacteriostatic substance in vitrain in English coals. He named it vitricin because of its occurrence in the vitrainous parts of coal seams. He demonstrated that it inhibited *Bacillus subtilis* and attributed differences in prevalence of pneumoconiosis among mine workers to differences in the amount of vitrain coal in different mines: the higher the percentage of vitrain coal in a mine, the lower the incidence of pneumoconiosis among the miners.

Vitrains from southern and northern Illinois and Wyoming coals were examined by a chromatograph technic similar to that used by Evans. The coal from northern Illinois was No. 2 coal, C rank, from Kankakee County, that from southern Illinois was No. 6 coal, B rank, from Franklin County, and that from Wyoming was a Hanna Basin coal. The examination of these coals revealed that all of them contained soluble material fungistatic in nature (Table 1). Subsequently R. M. Kosanke (*3*) of the Illinois State Geological Survey modified Evans's extraction technic in several ways and extracts obtained by these methods were tested for their fungistatic capacities. The method

TABLE. 1. Inhibition of *Endoconidiophora fagacearum* by extracts of vitrains. Measurements represent the maximum widths and lengths of the areas of inhibition around the extract-treated filter paper strips.

Origin of coal	Clear area measurements	
	Width (mm)	Length (mm)
Illinois Coal—Franklin County	11	58
Illinois Coal—Kankakee County	6	20
Wyoming Coal—Hanna Basin	17	76

used in obtaining the extract reported on below is briefly as follows. Vitrain, after being ground into particles of small size, is washed several times with a mixture of equal parts of acetone and methanol. The liquid resulting from this elution is then poured into a Coors porcelain evaporating dish. A sheet of Whatman No. 1 filter paper $2\frac{1}{4} \times 5\frac{1}{2}$ in. is suspended so that about $\frac{1}{2}$ in. of the paper is submerged in the liquid. The liquid is absorbed by the filter paper and, as the solvents evaporate from the filter paper, a chromatograph is produced that has several distinct bands of light and dark brown.

Two such chromatographs developed from Hanna Basin vitrain coal of Wyoming were tested against eight fungi: *Aspergillus niger, A. terreus, Myrothecium verrucaria, Glomerella cingulata, Colletotrichum trifolii, Verticillium albo-atrum, Endoconidiophora fagacearum,* and *Ceratostomella ulmi. A. niger, A. terreus,* and *M. verrucaria* are saprophytes. *G. cingulata* causes bitter rot of apples; *C. trifolii,* anthracnose of clover. The remaining three are vascular parasites of plants; *E. fagacearum* and *C. ulmi* being highly virulent pathogens respectively of oak and elm, *V. albo-atrum* a destructive pathogen of various trees, shrubs, and herbs.

To test the potency of the material deposited in the chromatograph the following procedure was used. Eight milliliters of potato dextrose agar, pH 5.6, were poured into a sterile Petri dish. After the agar had solidified, the surface was thinly flooded with a suspension of spores obtained from young cultures grown on test tube slants of potato dextrose agar, pH 5.6, at 25° C. A strip $2\frac{1}{4}$ in. wide was cut from the top of the chromatograph, as it contained none of the fungistatic material, and the remainder, $3\frac{1}{4}$ in. long, was cut vertically into strips 3/16 in. wide. After allowing sufficient time for the water of the spore suspension to be absorbed by the agar, a strip chosen at random was placed on the surface of the agar. The Petri dish was then incubated at 25° C and observed at intervals. The test for each fungus was run in triplicate.

That the extract in the chromatograph inhibited spore germination was shown by the maintenance on either side of the chromatograph strip of clear areas into which the fungistatic material had diffused in sufficient amount to prevent germination of the spores. The diagrams, Fig. 1, show the patterns of inhibition

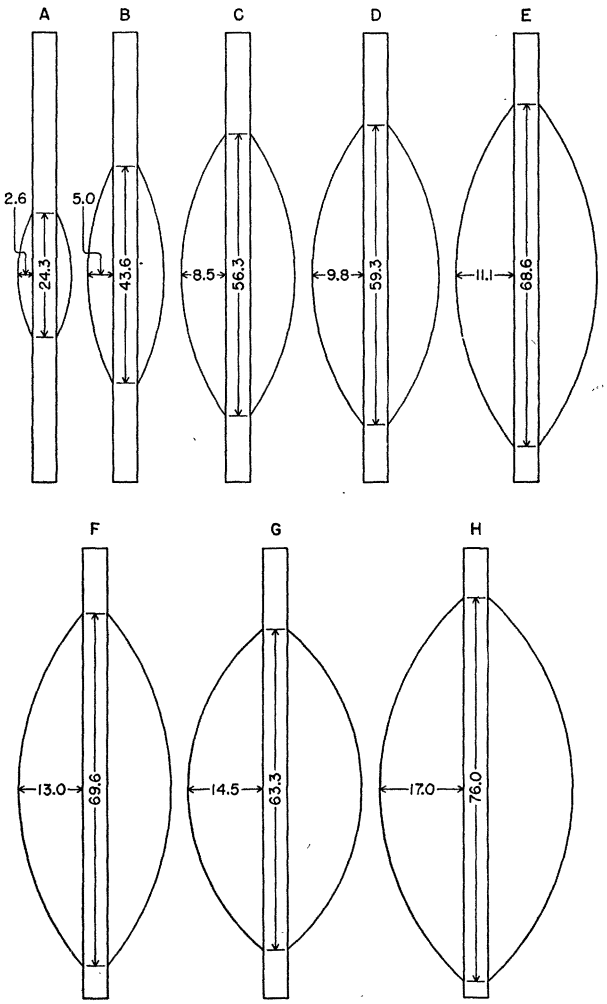

FIG. 1. Diagrams of the average maximum zones of inhibition around extract-impregnated filter paper (from Wyoming coal—Hanna Basin). A. *A. niger* in 24 hours; B. *A. terreus* in 24 hours; C. *M. verrucaria* in 33 hours; D. *V. albo-atrum* in 43 hours; E. *G. cingulata* in 33 hours; F. *C. ulmi* in 33 hours; G. *C. trifolii* in 43 hours; H. *E. fagacearum* in 74 hours.

for the eight fungi. The measurements are the averages of the widths and lengths of three replications when inhibition was at a maximum. *A. niger* was inhibited least, *E. fagacearum* most. Inhibition of the other fungi lay between these extremes. The greatest width of inhibition usually occurred about 27 mm above the part of the chromatograph that had been submerged. At this point there was a band in the chromatograph which appeared translucent when placed in front of a light.

Because of differences among the test fungi in time required for spore germination and in rate of growth of hyphae, different lengths of time, for example, 24 hr for *A. niger*, 74 hr for *E. fagacearum*, were required for the maximum, or first, area of inhibition to become defined. Such differences no doubt influenced the size of the regions of inhibition, as the slower fungi would afford opportunity for wider diffusion of the fungistatic material. Once established, however, the inhibition area usually remained constant for 12 or more hours, after which invasion from the outer margin occurred, *A. niger* advancing 1 mm, *A. terreus* 21 mm, in 54 hr.

The effect of the extract was fungicidal on *E. fagacearum* and *C. trifolii*, but fungistatic on the other six fungi. This difference in effect may have been due, also, to the rate of spore germination, as the spores of *E. fagacearum* and *C. trifolii* required longer periods for germination and were therefore exposed longer to the extract than were the spores of the other fungi.

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A Bacteriostatic Substance Extracted from the Vitrain Ingredient of Coal[1]

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A substance obtained from vitrain (bright bands in banded coal) can prevent the growth of *Bacillus subtilis*, as reported by Evans (*1, 2*). He termed this substance vitricin. To extract the substance, Evans covered finely ground vitrain with methanol. Strips of Whatman No. 1 filter paper were suspended about ½ in. into the mixture of methanol and coal, and the active material, vitricin, was carried along with the solvent in this modified ascending paper chromatograph method.

A bacteriostatic substance has been extracted from American coals using the method proposed by Evans and by other methods and with other solvents. This substance has been extracted (see Figs. 1, 2) from lignite, subbituminous coal, and high volatile bituminous coal of C, B, and A rank (classification of coal according to the degree of metamorphism from lignite to anthracite). The bacteriostatic action of extracts from high volatile A rank coal is slight, and entirely absent from one sample of the Buck Mountain anthracite. It would seem, therefore, that either the substance is absent or it cannot be extracted from higher rank coal, at least with present methods.

A substance extracted from a Pleistocene wood and two modern woods prevents the growth of *B. subtilis*. Whether this substance is the same as that obtained from coal is not known.

Initially, Evans' method was employed for the extraction of the substance from vitrain of Illinois Herrin (No. 6) coal. A bacteriostatic substance was adsorbed on paper strips, as shown in Fig. 1D. The

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