counteracted by auxin, namely *trans*-cinnamic acid, which was shown by van Overbeek (*10*) to have this interesting characteristic. Apparently its activity comes from its being an isomer of a compound showing growth-regulator activity. Maleic hydrazide, however, must not derive its activity in such a direct manner, because it has no side chain or acid group such as are required for growth regulators (*4*).

References

1. SCHOENE, D. L., and HOFFMAN, O. L. *Science,* **109**, 588 (1949).
2. JOHNSON, E. L. *Plant Physiol.,* **11**, 319 (1936).
3. SKOOG, F. *J. Cellular Comp. Physiol.,* **7**, 227 (1935).
4. WENT, F. W., and THIMANN, K. V. *Phytohormones.* New York: Macmillan (1937).
5. THIMANN, K. V., and SCHNEIDER, C. L. *Am. J. Bot.,* **25**, 627 (1938).
6. LEOPOLD, A. C., and KLEIN, W. H. *Plant Physiol.* (in press).
7. GALSTON, A. W. *Am. J. Botany,* **34**, 356 (1947).
8. VELDSTRA, H., and HAVINGA, E. *Rec. trav. chim.,* **62**, 841 (1943).
9. BONNER, J. *Am. J. Botany,* **36**, 429 (1949).
10. OVERBEEK, J. VAN, BLONDEAU, R., and HORNE, V. *Ibid.* (in press).

A Qualitative Analysis of Capsular Polysaccharides from *Cryptococcus neoformans* by Filter Paper Chromatography

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The technique of filter paper partition chromatography (*1*) has been shown to provide a convenient method for the determination of sugars present in a mixture (*2*) or of monosaccharide components of polysaccharides following hydrolysis (*3*).

This procedure has been applied to a qualitative analysis of the capsular polysaccharide which numerous investigators (*4–10*) have isolated from the pathogenic yeast, *Cryptococcus neoformans.* Aside from general qualitative chemical tests for carbohydrates, there have been no studies on the chemical composition of this polysaccharide.[3]

A representative polysaccharide was chosen from each of the three antigenic types (A, B, and C) of *C. neoformans* (*5, 11, 12*). The polysaccharides were isolated from neopeptone dialysate broth cultures by alcohol precipitation. Details of the method used for purification are presented elsewhere (*5*).

Twenty-mg samples of each polysaccharide were hydrolyzed in 0.5 ml 1 *N* H_2SO_4 for 2.5 hr at 100° C. Neutralization of the hydrolysate was then effected by slowly adding solid barium carbonate. The precipitate was removed by centrifugation, and the supernatant fluid was applied to filter paper sheets for the analysis.

In performing the tests, strips of Whatman No. 1 filter paper 17.5 cm × 30–40 cm were employed for descending chromatograms. The lower end of each sheet was cut to a point to facilitate drainage of the solvent (*13*). Two solvents were employed with this method, *n*-butanol saturated with acetic acid (10%) and water (50%), as described by Partridge (*2*); the second solvent was ethyl acetate containing acetic acid and water in a 3 : 1 : 3 mixture (*13*).

TABLE 1

RELATIVE MIGRATION DISTANCES (R_F AND R_X VALUES) FOR HYDROLYZED POLYSACCHARIDES AND FOR REFERENCE MONOSACCHARIDES USING 4 DIFFERENT SOLVENTS

Reference monosaccharides and hydrolyzed polysaccharides	Solvents			
	Ethyl acetate (R_X)	*n*-Butanol (R_X)	Acetone (R_F)	Phenol (R_F)
D (+) xylose	1.00	1.00	0.67	0.47
L (−) fucose	1.07	1.22	0.68	.65
L (−) rhamnose	1.12	1.34	—	.61
D (−) ribose	1.11	1.16	0.72	.62
L (+) arabinose	0.95	0.90	.62	.56
D (+) galactose	.70	.71	.48	.46
D (+) glucose	.73	.77	.53	.42
D (+) mannose	.79	.86	.58	.51
D (−) fructose	.85	.88	.58	.55
D (+) glucosamine HCl	*T**	.69	*T*	.63
D (+) galacturonic acid	.67	.47	.15	.13
D (+) glucuronic acid	0.68	0.46	0.20	0.12
Type A hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.48 *T*	0.51 0.47 *T*
Type B hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.49 *T*	0.51 0.47 *T*
Type C hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.49 *T*	0.51 0.47 *T*

* *T* = trailing spot for which no value was calculated.

On a pencil line drawn across the paper 2.5 cm from the edge of the trough, spots of the polysaccharide hydrolysates were placed with a micropipette calibrated to deliver 4 µl. Spots of the hydrolysates and spots of known reference sugars were placed along the line at intervals of 2 cm. The concentration of the hydrolysates was increased by adding a spot, allowing it to dry, and applying another spot at the same point. The sugars employed for reference are indicated in Table 1.

The filter paper strips were irrigated with solvent

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[3] Subsequent to the writing of this manuscript, personal communication with E. Drouhet and G. Segretain (*4*) has disclosed that they have recently submitted a paper in which it was shown that xylose, mannose, and a uronic acid were present in the polysaccharide with which they are working.

in the usual manner (*1, 2, 13*). Trial chromatograms with *n*-butanol disclosed that excellent separation of sugar spots was obtained in 36–48 hr. This time was adjusted so that the most rapidly migrating sugar remained well above the lower end of the chromatogram. The papers irrigated with ethyl acetate were allowed to run for 24–30 hr. After irrigation, the solvent was removed from the chromatograms by evaporation in a circulating air oven at 85° C. The sugar spots were located by spraying with the silver nitrate reagent of Partridge (*2*) and developing in an oven at 85°–95° C.

Since the solvent was allowed to flow off the end of the paper it was not possible to calculate the R_F value (*2*). Instead, the value "R_X" was used. With this system, xylose was arbitrarily assigned a migration distance of 1.00, and the location of the other sugar spots was calculated proportionally.

Two additional solvents were employed in an ascending chromatographic method, the phenol-ammonia mixture of Partridge (*2*) and commercial acetone to which 10% water by volume was added. In these chromatograms, the filter paper sheets were rolled into cylindrical form and allowed to irrigate by the ascending technique of Williams and Kirby (*14*). Five to eight hours were sufficient for migration of the acetone and 10–12 hr for the phenol. Development of the spots was conducted as with the other solvents. R_F values were calculated for these chromatograms by the usual method (*2*).

The separation of the components of the hydrolysates was not equally good with all the solvents. Results with the *n*-butanol and ethyl acetate were more satisfactory than with the other two solvents. This was partly due to the better separation possible with the modified descending technique (*13*) used with the *n*-butanol and ethyl acetate.

An examination of the R_F and R_X values in Table 1 reveals that the polysaccharides of all three types exhibited spots migrating similarly to xylose, mannose, and galactose.

In chromatograms irrigated with butanol, mannose was differentiated from fructose by spraying with a resorcinol-HCl mixture (*15*). The fructose control produced a bright red spot when this reagent was employed, but the mannose spot was barely visible.

Each hydrolysate possessed an additional slower-moving spot in the region of galacturonic acid and glucuronic acid. This spot did not coincide exactly with either of the two reference uronic acids, however, and since additional uronic acids were not available the spot has not been identified. It seems likely that this spot represents the uronic acid responsible for positive Dische tests (*4, 5*). The uronic acid spot was well isolated only with the butanol-acetic acid mixture. It formed a trail when phenol and acetone were employed as solvents.

The hydrolysates of polysaccharides from each of the three antigenic types of *C. neoformans* are qualitatively similar. The fact that all three polysaccharides contain the same four monosaccharides offers some insight into the close antigenic relationships among the three types (*5, 11, 12*).

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, **38**, 224 (1944).
2. PARTRIDGE, S. M. *Ibid.*, **42**, 238 (1948).
3. *Ibid.*, 251.
4. DROUHET, E., and SEGRETAIN, G. *Rev. path. comp. et hyg. gen.*, **50**, 37 (1950).
5. EVANS, E. E., and KESSEL, J. F. (In press.)
6. HEHRE, E. J., CARLSON, A. S., and HAMILTON, D. M. *J. Biol. Chem.*, **177**, 289 (1949).
7. KLIGMAN, A. M. *J. Immunol.*, **57**, 395 (1947).
8. MAGER, J. *Biochem. J.*, **41**, 603 (1947).
9. NEILL, J. M., *et al. J. Exptl. Med.*, **89**, 93 (1949).
10. STANLEY, N. F. *Australian J. Exptl. Biol. Med. Sci.*, **27**, 409 (1949).
11. EVANS, E. E. *Proc. Soc. Exptl. Biol. Med.*, **71**, 644 (1949).
12. ———. *J. Immunol.*, **64**, 423 (1950).
13. JERMYN, M. A., and ISHERWOOD, F. A. *Biochem. J.*, **44**, 402 (1949).
14. WILLIAMS, R. J., and KIRBY, H. *Science*, **107**, 481 (1948).
15. FORSYTH, W. G. C. *Nature*, **161**, 239 (1948).

The Relationship of Muscle Potassium to the Melanophore-concentrating Effect of Pressure on the Trout[1]

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Observant fishermen have noted that not only do trout lose their brillant coloring at varying intervals of time after being taken from the water but also that the pallor is often patchy and occurs principally at points of pressure. The patterns of leaves or ferns used for packing the creel are frequently sharply outlined on the trout's skin. Fish kept for relatively long periods tend to become uniformly pale. This latter type of pallor is very likely due to the melanophore-concentrating effect of temperatures above those to which the trout is accustomed (*1*). That caused by pressure has not been explained satisfactorily. The only investigator who has apparently paid any attention to this phenomenon is von Frisch (*2*). He concluded that concentration of the melanophores from pressure was due to the local accumulation of acid products of metabolism. The experimental findings outlined in the present communication point to another mechanism.

Observations on the effects of pressure applied to the body surface of the trout under varying conditions have been described in a previous study (*1*). The salient findings were as follows:

1. A rainbow trout (*Salmo gairdnerii*), killed instantly by a sharp blow on the head and laid on a hard surface, exhibited pallor of the underside within 20–30 min. Turning the fish over resulted in a gradual dispersion of the concentrated melanophores, and those of the side now underneath concentrated. This reversal phenomenon, dark to pale and pale to dark, could be induced repeatedly. Pallor from pressure

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