from fungal noncellulosic glucans were similar to the spectrum of laminarin; an absorption at 1072 cm^{-1} occurs in laminarin and both fungal glucans (Fig. 2). The second principal absorption appears at 1042 cm^{-1} in the *Pythium* spectra, and the corresponding laminarin absorption occurs at 1040 cm^{-1}. We do not suggest that these observations alone provide a basis for distinguishing between different types of cell-wall polysaccharides, but they do constitute corroborative evidence for glucan heterogeneity in oomycete walls. Moreover, it should not be inferred that there is unqualified similarity in chemical constitution between laminarin and the fungal glucans, since mannitol is a known constituent of the former (*17*) and apparently is absent in the latter (*6*).

Our investigation, along with that of others (*4, 5*), therefore, reveals a significant coherence among selected Oomycetes with respect to the structure of their cell walls. The walls of other Oomycetes in the orders Leptomitales and Lagenidiales have not been investigated in detail in any instance. Since criteria based on morphology have led to the belief that members of these little-investigated taxa are closely allied with the fungi that we and others have examined, one could predict features of the composition of cell walls in these other groups. On the other hand, more complete quantitative investigations (*5–7*), employing mechanically isolated walls, show that studies such as ours do not demonstrate all the salient features of cell-wall structure. Therefore, investigations of the structure of cell walls in the Leptomitales and Lagenidiales are clearly warranted.

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9. We used chemically isolated walls in the present study for convenience. Mechanically isolated walls of *Atkinsiella* and *Pythium* (see *6* and *7*) yielded polysaccharide fractions with the same properties as fractions obtained from chemically isolated walls.
10. We thank W. Z. Hassid for a sample of authentic laminaribiose, H. J. Potgieter for a sample of β-(1→3)-glucan glucanohydrolase, R. B. Park for giving us access to equipment for infrared analyses, and B. J. D. Meeuse for a generous supply of laminarin.
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Iodide Transport: Inhibition by Agents Reacting at the Membrane

Abstract. *Accumulation of iodide by thyroid tissue is inhibited by two phospholipase A-free proteins from cobra venom, filipin, crude phospholipase C, and lysolecithin. The venom proteins decrease K^+ in tissue but do not significantly affect incorporation of phosphorus-32 into phospholipid or stimulation of this process by thyrotropin. However, filipin and crude phospholipase C, like thyrotropin, do increase phospholipid formation.*

Phospholipids may play an important role in transport processes across cell membranes. In the thyroid, a possible carrier role of phospholipid (*1*) has been proposed, and sensitivity of the synthesis of phospholipid to such membrane-active reagents as digitoxin (*2*) and thyrotropin (TSH) (*3*) has been demonstrated. We therefore investigated the action of several substances, known to affect membrane phospholipids, on I^- transport into beef thyroid slices. Brief preincubation with crude venom from the cobra (*Naja naja*) as a source of phospholipase A, crude phospholipase C (from *Clostridium perfringens*), lysolecithin, and filipin—a polyene antibiotic—all blocked the subsequent accumulation of $^{131}I^-$ by thyroid slices. Column chromatography of cobra venom showed that the inhibiting activity was independent of phospholipase A but was associated with two distinct, late-eluting protein fractions. The effects of these proteins and the above agents on accumulation of I^-, incorporation of ^{32}P into lipid, and concentration of K^+ in tissue, have been compared.

Phospholipase A activity was determined by titration according to the method of Rodbell (*4*), but at a *p*H of 7.7 and a temperature of 24°C. Accumulation of iodide in beef thyroid slices was measured as previously described (*5*); formation of lipid-^{32}P and the effect of TSH (20 units/mg) were determined according to Kögl and van Deenen (*3*). Tissue cations were determined by flame photometry after heating the tissue in 1.0*M* H_2SO_4 for 15 minutes at 100°C. Fractionation of lyophilized *N. naja* venom on carboxymethyl cellulose was performed by a modification of the method of Yang *et al.* (*6*). Rechromatography of the concentrated eluates was carried out on the same column and yielded two active fractions which we labeled proteins A and B.

Slices were preincubated with the inhibitors in Krebs-Ringer phosphate buffer at 37°C for 15 to 30 minutes. In some experiments 3 percent albumin and additional Ca^{++} were present. Filipin was added in dimethyl sulfoxide. Inhibitors were removed by decanting, and the slices were rinsed twice with 5 to 10 ml of 0.154*M* NaCl and once with 5 to 10 ml of Ringer-phosphate medium; they were then incubated for 15 minutes in Ringer-phosphate medium to complete the wash. All subsequent tests were carried out on such slices.

Except for purified phospholipase A, all the compounds tested interfered with accumulation of iodide by thyroid slices (Table 1). Proteins A and B were approximately two to four times as potent as crude venom but contained < 0.1 percent of the phospholipase ac-

tivity; protein B was somewhat more potent than A. Addition of purified phospholipase A to either protein (A or B) did not enhance their capacity to inhibit accumulation of iodide.

Crude phospholipase C had about the same potency as crude cobra venom, although there was a twofold range between different commercial preparations. It remains to be shown whether this activity is associated with phospholipid hydrolysis. Filipin was a potent inhibitor of the mechanism for concentrating iodide. A similar effect has been described for amphotericin B (*7*). At a $2 \times 10^{-4}M$ concentration, lysolecithin inhibited the accumulation of iodide by 50 percent. Unlike the effect of filipin, the inhibition by lysolecithin was readily prevented by incubating the slices in an excess of albumin, the prevention presumably being due to binding by this protein (*4*). The inhibitory effects of protein A could be demonstrated after preincubation for as little as 1 minute. With longer preincubation, an optimum time (approximately 15 minutes) existed for maximum inhibition. Thereafter, there was progressive recovery of the ability to concentrate I^-.

During preincubation of thyroid tissue with protein B and the subsequent washing, a 30-percent decrease occurred in the concentration of K^+ in the slices (Table 2). Despite loss of K^+ in the tissue produced by protein B, increasing the concentration of K^+ in the medium to 15 or 25 meq/liter during preincubation or subsequent incubation failed to protect the thyroid slices against depression of the capacity to concentrate iodide. Filipin caused a similar loss of K^+ (Table 2). In contrast, crude preparations of phospholipase C led to little change in concentrations of K^+ in thyroid slices under conditions that caused a 50- to 70-percent decrease in the ability to concentrate iodide.

To ascertain whether these effects constituted specific changes in electrolyte metabolism or represented nonspecific tissue damage, we investigated the effect of these agents on incorporation of ^{32}P into thyroidal phospholipids. Since this system is sensitive to TSH in vitro, responsiveness to this hormone offered an additional test of tissue integrity. Amounts of protein B that caused a 60-percent reduction in the capacity to concentrate iodide did not affect basal incorporation of ^{32}P into the lipid fraction and had only a slight effect on the ability to respond to TSH with an increase in the formation of ^{32}P-phospholipid (Table 3).

In contrast, crude phospholipase C caused a marked increase in base line incorporation of ^{32}P into the lipid fraction of thyroid slices. Such slices also have a reduced capacity to increase further the formation of lipid-^{32}P under the influence of TSH. These results are similar to the effect on glucose uptake in the isolated fat cell (*4*). However, it seems premature at this time to ascribe identical mechanisms to the effects of crude phospholipase C and TSH for the following reasons: (i) The phospholipid species that was stimulated has not been identified and may well be different. (ii) The crude preparation of phospholipase C, like crude venom, contains numerous enzymes, any one of which could cause this effect. (iii) A similar effect is shared by an entirely different agent—filipin. The occasional thyroid gland in which accumulation of I^- was not affected at these doses of crude phospholipase C still showed the same two effects on formation of lipid-^{32}P, albeit to a lesser extent (Table 3). Filipin produced changes in incorporation of ^{32}P into lipid quite similar to those produced by the crude phospholipase C.

Despite the fact that accumulation of I^- was decreased by these agents, their effect on incorporation of ^{32}P into phospholipids or concentration of K^+, or both, was markedly different. Thus, crude phospholipase C and filipin increased formation of lipid-^{32}P. By contrast, the inhibitory proteins from *N. naja* venom did not influence either synthesis of phospholipid or its stimulation by TSH. On the other hand, filipin and crude phospholipase C differed in their effect on the concentration of K^+ in tissue. While all three agents probably act on the thyroid cell membrane, differences in the responses of associated phospholipid and K^+ suggest that the effects on I^- transport are produced by different mechanisms.

Table 1. Inhibition of accumulation of iodide in beef thyroid slices by various agents. Figures in parentheses are numbers of separate glands tested. All determinations were performed in triplicate. Abbreviations for agents are: CV, crude *N. naja* venom; PLA, phospholipase A fraction; PrA, protein A; PrB, protein B; CPLC, crude phospholipase C; F, filipin; and LL, lysolecithin.

Agent	Relative activity of phospholipase A*	Preincubation with toxin (min)	Conc. for 50% inhibition (μg/ml)
CV (13)	1	30†	15 to 30
PLA (4)	3	30†	$\gg$ 240
PrA (8)	0.0009	30†	10 to 20
PrB (15)	.0007	15	10 to 20
CPLC (4)		15	30 to 50
F (8)		30	10 to 15
LL (2)		30	$2 \times 10^{-4}M$

*Based on activity of crude *Naja naja* venom being 1. †Preincubation in the presence of 3 percent bovine serum albumin.

Table 2. Concentration of K^+ (expressed as percentages of the controls) in beef thyroid slices after exposure to toxins for 30, 60, and 120 minutes. During the initial 15 minutes the tissue was incubated with toxin at a concentration that gave 50- to 70-percent inhibition of I^- accumulation. Values are averages of quadruplicate determinations on at least two separate tissues.

Toxin	Conc. of K^+ (% of control) after (min)		
	30	60	120
N. naja protein B	60	73	66
Filipin	88	69	62
Phospholipase C	88	91	104

Table 3. Incorporation of ^{32}P into the lipid fraction of beef thyroid slices. After preincubation, slices were incubated with or without TSH for 3 hours at 37°C in Krebs-Ringer bicarbonate buffer containing 0.0012M KH_2PO_4. Calculations are the averages of triplicate determinations based on the final wet weight.

Inhibition of accumulation of I^- (%)	Basal incorporation of ^{32}P (% of control)	Stimulation of ^{32}P incorporation by TSH	
		Without toxin (%)	After toxin (%)
Protein B (25 to 30 μg/ml)			
60	102	191	175
Crude phospholipase C (40 μg/ml)			
0	129	184	146
49	221	214	148
Filipin (50 μg/ml)			
71	202	193	119

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