NaOH, and 0.1N HCl. The precipitate was also lyophilized, and attempts to extract the color were fruitless.

None of the following drugs at $5 \times 10^{-4}M$ produced the blue color with crude mitochondria: amphetamine HCl, adrenalin, bufotenine bioxalate, bulbocapnine HCl, chlorpromazine HCl, harmine HCl, LSD 25, or serotonin creatine–H_2SO_4. Formation of the colored complex seems quite specific for psilocybin.

Several compounds were tested for their individual effect on the production of the blue complex. Psilocybin, crude mitochondrial homogenate, and the test compound at the same concentration as the psilocybin ($2.5 \times 10^{-4}M$) were mixed and shaken overnight. Harmine, ouabain, atropine sulfate, *d*-tubocurarine chloride, bufotenine, bretylium tosylate, reserpine, chlorpromazine, sodium pentobarbital, serotonin, and tyrosine had no effect but production of color was about 80 percent inhibited by ethyldiaminetetraacetic acid (EDTA) or tyramine HCl and less inhibited by 2,4-dinitrophenol.

Two questions remain: Has this phenomenon any bearing on the hallucinogenic action of psilocybin? By what mechanism is the color formed? Evidence against a relation to hallucinogenesis is the high concentration required, and the fact that the closely related hallucinogen bufotenine (which has a 5-hydroxyl rather than a 4-phosphate substituent) gives no blue color. However, with respect to the concentration aspect, it is of course possible that the blue color is an extreme manifestation of an effect whose more-subtle consequences, at much lower concentrations of psilocybin, may suffice to affect brain function.

In discussion of mechanisms, two related observations may be considered. Ehrenpreis *et al.* (*7*) found that several catecholamines combine with the highly phosphorylated egg protein, phosvitin, to give a blue color whose formation is blocked by tyramine or EDTA (*8*). Can it be that a phosphate group is needed—in our case provided by the psilocybin? This idea would explain why bufotenine is ineffective in our system. The phosvitin effect increased with *p*H, as did the psilocybin effect. Maas and Colburn (*9*) found that nerve-ending particles and synaptic vesicles contain appreciable amounts of magnesium, copper, and iron; they suggested that the metals in these components form a ternary complex with adenine triphosphate (ATP) and norepinephrine; complex formation is blocked by the chelating agent ethylenediamine. Perhaps in this instance a phosphate requirement is supplied by ATP. The interference reported by us for 2,4-dinitrophenol may result from an effect on endogenous ATP.

There remains a question of whether the formation of blue by psilocybin is connected with norepinephrine binding. Herblin (*2*), after using well-washed crude mitochondrial fraction from rat brain, reported that norepinephrine as well as psilocybin was required for the blue color; but in our unwashed preparation we found no such effect. Yet the interference by tyramine is suggestive, because tyramine is believed to interfere with norepinephrine binding in a special pool from which it is released by nerve impulses (*10*). Finally, psilocybin is reported (*2, 11*) to promote the uptake of norepinephrine by crude rat-brain mitochondria.

The *p*H dependence of the formation of psilocybin blue is compatible with involvement of the unprotonated indole group of psilocybin. The inactivities of bufotenine and serotonin suggest that a phosphate group also is required. It remains to be seen whether additional features of the psilocybin molecule are required for the effect.

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Abductin: A Rubber-Like Protein from the Internal Triangular Hinge Ligament of Pecten

Abstract. *The rubber-like internal triangular hinge ligament from* Pecten *was studied by light and electron microscopy, x-ray diffraction, and chemical analysis. The ligament is composed of an amorphous protein, abductin. In physical properties abductin is similar to elastin and resilin but distinct by amino acid analysis. It is characterized by high concentrations of glycine and methionine.*

During investigation of the adductor muscles of scallops, a structure that felt like vulcanized rubber was encountered in the hinge. The scallop swims by means of a bellows-like action of the valves, and the internal triangular hinge ligament (ITHL) acts as an antagonist to the adductor muscle. When the valves are closed by contraction of the adductor muscle this ligament is compressed, and upon relaxation of the muscle the elastic spring-like action of the ligament causes the valves to abduct.

Our preliminary investigations and the results of other investigators showed that the hinge ligament has properties similar to those of the other known elastic proteins, elastin and resilin. Trueman (*1*) estimated the modulus of elasticity for the ITHL of *Pecten* to be 3×10^7 dyne/cm^2. Alexander (*2*), correcting for creep, estimated Young's modulus to be 4×10^7 dyne/cm^2. Both investigators measured compression of the ligament. In our measurements of stretch of ITHL with an Instron strain gauge, the modulus is estimated to be 1.25×10^7 dyne/cm^2. Young's modulus for elastin is estimated to be 6×10^6 (*3*) while for resilin it is 4×10^7 dyne/cm^2 (*4*). Alexander concluded from his thermodynamic calculations that the elasticity of the ITHL is true rubber-like elasticity, in that elastic force is associated mainly with entropy changes (*2*). Like resilin and elastin, the ITHL is rubber-like only when it is hydrated and is rigid and leather-like when dry.

We studied the ITHL by light and electron microscopy, x-ray diffraction, and chemical analysis in order to compare the properties of this elastic ligament with those of elastin and resilin.

Ligaments from *Placopecten magellanicus* and *Aequipecten irradians* were dissected away from the valves, and

the calcareous attachment plate was trimmed from either end, leaving a dark, noncalcareous rubber-like, triangular prism. These ligaments were stored air dried or in filtered sea water at 0°C, or the ligament was frozen and stored at −20°C.

Histological sections stained with hemotoxylin and eosin show that the ligament is acellular; it is arranged in a dense, lamellar, cortical region and a loose trabecular medullary portion. The interstices of the medullary region appear empty; the compact lamellae of the cortical region run generally parallel to one another. The ligament was difficult to section for electron microscopy because the tissue was not readily penetrated by Vestopal. Sections were obtained, however, from small pieces of the ligament that had adequate peripheral support. In these sections it appears as an acellular, homogeneously amorphous substance of low electron density.

With high- and low-angle x-ray diffraction no distinct patterns were obtained either in the stretched and unstretched dried state or in the stretched and unstretched wet state. Thus observations made by microscopy and x-ray diffraction indicate that the ITHL is amorphous. These results, combined with the rubber-like elasticity, are similar to those obtained with resilin (*5*).

Kjeldahl analysis of the ITHL gave a determination of 12.9 percent total nitrogen. With 16 percent as a standard for protein, the ligament is 80.6 percent protein; it was quickly digested by pepsin but proved insoluble in all the protein solvents used. These included 6*M* urea, formamide, saturated guanidine hydrochloride, formic acid, and alkaline thioglycolate. Swelling was observed after 2 hours in 6*M* urea, in 1 percent thioglycolate, and in saturated guanidine hydrochloride. When it was tested by the Lowry extraction method (*6*) for elastin, which consists of treatment with 0.1*N* NaOH at 98°C for 1 hour, the ligament was completely hydrolyzed, indicating that the protein is unlike elastin. The lipid determination, which involved a 1-hour reflux in 4*M* NaOH followed by three extractions with ethyl ether, showed no residue in the dried flask and no measurable weight change. Tests for glucosamine and galactosamine also were negative. The Molisch reaction (*7*) yielded a red-violet color with an absorption maximum at 570 mμ. Based on ribose the material contains 0.9 percent pentose, and on glucose 0.6 percent hexose. One sample of ITHL was ashed overnight at 450°C, and the ash content was 2.8 percent; of this, 20 percent was Ca, as determined by emission spectroscopy.

For amino acid analysis, the ligament was ground with mortar and pestle and predried under reduced pressure at room temperature for 24 hours. A weight loss of 5.8 percent occurred. The sample was hydrolyzed in 6*N* HCl at 110°C for 22 hours in a sealed, evacuated ampule. Determination of the amino acids in the hydrolyzate followed the standard procedure of the Spinco amino acid analyzer, model 120. This analysis is given in the first four columns of Table 1. Averages of duplicate or triplicate analyses are reported for abductin. For comparison, Table 1 also gives the composition of other proteins that contain large amounts of glycine. All are expressed as residues per 1000 total residues regardless of their original tabulation.

Compositions of ITHL of *Placopecten magellanicus* and of *Aequipecten irradians* appear to be very distinct from both resilin and elastin, and, although there are individual variations in the concentration of amino acids in these two species, the similarity in high concentrations of glycine and methionine is striking. The high concentration of methionine is interesting since little methionine has been reported in the other known elastic proteins. Considering the principles of elasticity, we would expect a relatively high degree of cross-linking between peptide chains. Since the usual amino acids involved in cross-linkages are absent or of low frequency, we examined the possibility that a dimer of methionine or similar amino acid containing a disulfide bridge might chromatograph under the methionine peak. Authentic samples of lanthionine and cystathionine were added to aliquots of standard amino acid calibration mixture and were readily detected separate from methionine. In addition, paper chromatography of these amino acids, along with a hydrolyzate of ITHL, showed that lanthionine and cystathionine were not

Table 1. Amino acid residues per 1000 residues. Abbreviations: Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; ½ Cys, half cystine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Lys, lysine; His, histidine; Arg, arginine; Hyp, hydroxyproline; Hyl, hydroxylysyl.

Amino acid	ITHL (whole)		ITHL cortex (*Placopecten magellanicus*)	ITHL medulla (*Placopecten magellanicus*)	Resilin (*13*) (Wing hinge, *Schistocera gregaria*)	Elastin (*14*) (*Ox ligamentum nuchae*)	Collagen (*15*) (calf skin)	Silk (*16*) (fibroin, *Bombyx mori*)
	Placopecten magellanicus	*Pecten irradians*						
Asp	69.9	18.1	69.8	72.0	102.0	4.0	48.1	12.1
Thr	7.4	11.3	6.7	6.5	28.0	7.0	19.4	9.1
Ser	36.4	68.8	34.5	38.0	80.0	7.0	36.9	121.1
Glu	19.4	12.0	19.2	17.6	47.0	13.0	74.7	10.6
Pro	7.4	12.6	7.5	7.9	77.0	143.0	126.0	3.8
Gly	620.3	629.9	622.5	621.9	385.0	364.0	324.0	446.6
Ala	26.5	32.9	23.9	24.1	111.0	194.0	109.0	294.5
½ Cys						Trace		
Val	3.5	6.0	2.5	2.7	28.0	135.0	26.4	22.7
Met	117.5	90.4	124.0	120.5	0	(<1)	7.1	0
Ile	4.0	7.5	3.7	4.0	17.0	28.0	13.2	6.8
Leu	2.9	1.8	2.7	2.5	23.0	60.0	28.3	5.3
Tyr	10.7	1.5	9.2	10.5	27.0	8.0	4.9	52.2
Phe	51.3	92.6	52.1	52.1	26.0	27.5	13.8	6.1
Lys	12.4	8.0	12.6	9.5	5.0	3.0	28.9	3.0
His	0.3	6.0	0.3	0.3	9.0	0.5	5.3	1.5
Arg	9.8	3.4	8.8	9.1	35.0	5.0	47.7	4.5
Hyp							85.6	
Hyl							6.8	

present in significant amount (*8*). Tryptophan was determined separately by the method of Spies and Chambers (*9*). Less than 1 residue per 1000 residues can be attributed to tryptophan.

The possibility that methionine hydrophobic bonds were strong enough to provide effective cross-links that give elastic properties to this insoluble protein has not been resolved. Prolonged exposure (2 weeks) to both iodoacetic acid and iodoacetamide (400 mg protein in 10 ml of 10-percent solution of either reagent at *p*H 2 and *p*H 4.5) at room temperature did not dissolve the ITHL although swelling was observed (*10*). Since the reaction between iodoacetic acid and methionine produces a charged residue, any hydrophobic bonds due to methionine would be disrupted. Homoserine and *S*-carboxymethylhomocysteine were detected in protein hydrolyzates treated with iodoacetic acid, and the methionine peak was diminished; however, further work must be done to determine the effect of these reagents on the methionine residues.

Histological sections showed a distinct morphological difference between the cortical and medullary portion of the ITHL. To determine if there was a difference in protein composition, the two regions were separated by dissection, and amino acid analyses were conducted on each. There is no difference in amino acid concentration in either region, which indicates that the protein is similar throughout the ITHL (Table 1). Treatment with ethylenediaminetetraacetate did not affect the amino acid composition of the ITHL of *Pecten* as it did the inner hinge ligaments of certain other bivalves that we studied (preliminary investigation). No large unknown peaks were found by column chromatography, although a few small unknown peaks were encountered spasmodically; none of these corresponded to desmosine. The possibility remains that material of unknown composition was bound tightly to the ion-exchange resin. The cross-linkages in resilin have been attributed to di- and tri-tyrosine, and Andersen found that these amino acids were held back on the ion-exchange column (*11*). However, these amino acids give rise to a "bright blue fluorescence" in ultraviolet light in intact resilin; in our initial attempts, intact ITHL does not appear to fluoresce (*12*).

These results indicate that the ITHL of *Pecten* is composed primarily of a rubber-like protein distinct from other known elastic proteins, and we propose the name abductin for this insoluble rubber-like protein. The name seems appropriate since the protein functions as the abductor of the valves of the scallop.

Any similarity in the properties of abductin from scallops and the proteins of the inner hinge of a wide range of bivalve mollusks has not yet been determined.

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Diphenamid Metabolism in Plants

Abstract. *Diphenamid, a herbicide, is metabolized to* N-*methyl 2,2-diphenylacetamide and 2,2-diphenylacetamide by the common soil fungi* Trichoderma viride *and* Aspergillus candidus *within 48 hours. The two metabolites are more toxic than diphenamid to both tomato and barnyard-grass seedlings under sterile conditions. This finding indicates that the phytotoxic moiety is not diphenamid but one of its metabolites—probably the* N-*methyl derivative.*

Diphenamid (*N,N*-dimethyl 2,2-diphenylacetamide) is an effective herbicide for controlling several annual grass and broadleaf species; it is absorbed through the roots and has negligible herbicidal activity when applied to the foliage (*1*). The absorption, translocation, and metabolism of ^{14}C-diphenamid has been studied in tomato plants (*2*). Seven days after treatment with diphenamid, both the MDA (*N*-methyl 2,2-diphenylacetamide) and DA (2,2-diphenylacetamide) metabolites were found in benzene extracts; after 21 days, no diphenamid was detectable in the plants. The *N*-demethylation of diphenamid in tomato plants was proposed as the mechanism of resistance by this species. *N*-Demethylation of methylamides and methylamines in both plants and animals is reported (*3*), but correlation between demethylation and phytotoxicity has not been established with plant species.

The fungi *Trichoderma viride* and *Aspergillus candidus* were cultured in 150-ml flasks containing 100 ml of half-strength Hoagland nutrient solution and 0.02*M* glucose. After 1 week, 0.036 μc of ^{3}H-diphenamid was placed in each flask. After 4- and 48-hour exposures, the diphenamid was extracted with chloroform and subjected to two-directional chromatography (type K301R Eastman thin-layer chromogram sheet), first in a benzene-ethanol (85:15 by volume) solution, and then in a benzene-diethylamine solution (95:5 by volume). The extract was cochromatographed with ^{3}H-diphenamid in the first solvent system and with diphenamid, MDA, and DA in the second.

The extracts exposed to either fungus for 4 hours contain a compound that migrates as MDA (R_F, 0.53) in the benzene-diethylamine system. There is also a spot corresponding to the diphenamid reference at R_F 0.64. Each extract after 48-hour exposure has a larger spot, corresponding to the MDA metabolite, at R_F 0.53, and also a detectable amount, at R_F 0.25, corresponding to the DA metabolite. Fur-