Table 2. Summary of data showing positive association between the Am 1 variant of serum amylase and the red cell antigen I_b in pigs. Data in Table 1 are included. Variant Am 3 was observed only in three Hampshire sows and 23 of their 41 progeny from six litters. All Am 3 pigs were I_b-positive.

Animals*	Distribution of phenotypes			
	A+ I_b+	A+ I_b−	A− I_b+	A− I_b−
H { Parents	33	0	83	68
H { Progeny	262	0	744	580
H Males	25	0	49	28
L,Y,L-Y Females	17	0	39	11

* H, Hampshire; L, Landrace; Y, Yorkshire; and L-Y, indicates L-Y crosses. † A+ and A− indicate presence or absence of Am 1 (or Am 3).

However, in the Danish Landrace breed, Am 3 was positively associated with I_a (*1*).

Apart from the exceptional progeny already considered, all data in Tables 1 and 2 are compatible with a hypothesis of pleiotropy—that is, a series of alleles at one locus each of which has two effects. For example, one allele might be responsible for an I_b-positive red-cell phenotype in conjunction with type Am 1 of serum amylase. Another allele might be responsible for an I_b-positive red-cell phenotype in conjunction with amylase type Am 3. Based on the present material, additional alleles, each with pleiotropic effect, may be visualized.

If pleiotropy rather than linkage is the correct interpretation of the data, then the observed exception to this hypothesis poses an interesting problem. A paternal mutation of I^a to I^b might be postulated because the boar had been exposed to gonadal x-irradiation, whereas the genetic material of the sow had not been exposed to x-rays (*10*). A change of Am^1 to "not Am^1" would require a spontaneous mutation during oögenesis, or such a change could be a result of gene interaction. If so, apparently only the enzyme variant was affected but not the blood factor determined by the same allele having pleiotropic effect.

Since the observations might have important theoretical implication, evidence on whether the ostensible amylase polymorphism is real or spurious is needed. According to Uriel (*11*) enzymes may be carried by, or form complexes with, other proteins or protein fragments. This possibility has been considered previously in connection with studies of amylase variations in pigs (*5*); because the three variants have not been observed in saliva and pancreas fluid (*6*), the latter is assumed to be the main source of serum amylase. Thus, amylase might be attached to, and follow, the migration of variants of a polymorphic system of other proteins in serum, and merely serve as an indicator for these proteins in the starch gel. Hence, the data show that the *I* blood-group locus and the *Am* locus for serum amylase, or possibly for amylase-binding constituents of serum, are closely linked or identical. The distinction between the two hypotheses depends on the correct interpretation of the phenotype of one exceptional pig out of the 1939 pigs considered.

The observations in pigs are comparable to the genetic association detected between the Ss (*12*) serum protein variants and the H-2 histocompatibility traits in the mouse (*13*). The *H-2* locus controls a set of red blood cell antigens and is also the strongest of several loci affecting tissue transplantation incompatibility. The Ss protein is a euglobulin, but tests for enzymatic activities have been negative. Although specific Ss and H-2 types always are associated, it is not clear whether the association reflects close linkage or multiple effects of the *H-2* locus.

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Isolation of Nuclei from a Marine Dinoflagellate

Abstract. *By means of a medium containing dextran, nuclei were isolated in high yield from cells of* Gymnodinium nelsoni, *a marine dinoflagellate. Most of the DNA, but less than one-tenth of the RNA, of the original cells was recovered in the purified nuclei. The nuclei appeared substantially intact as observed by light or electron microscopy. The isolated nuclei were capable of incorporating tritiated uridine triphosphate into material insoluble in cold acid. The general procedure was found to be applicable also to two species of diatoms.*

In order to test the generality of nucleic acid-mediated control systems hypothesized on the basis of studies on bacteria and bacterial viruses, it would be highly desirable to be able to obtain intact nuclei and other organelles from microorganisms that can be grown axenically, under controlled conditions, and in a defined medium. With few exceptions it has proved difficult to obtain intact nuclei from microorganisms.

We looked among the algae for species with large, naked cells and with a prominent nucleus. The large dinoflagellate (Fig. 1a) *Gymnodinium nelsoni* Martin (strain GSBL) (*1*) appeared the most promising, and we report here the characterization of nuclei obtained from this organism.

Subcultures of *Gymnodinium nelsoni*, maintained in algal culture at the Woods Hole Oceanographic Institution (WHOI), were grown with aeration and 11,000 lumens/m² of fluorescent

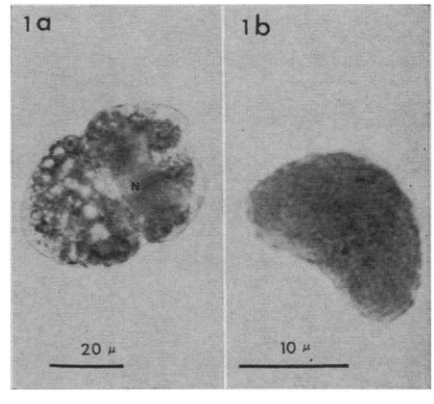

Fig. 1. (a) *Gymnodinium nelsoni* stained with methyl green in bright-field illumination. The nucleus is the lobed, opaque structure in the lower half of the cell. (b) Isolated nucleus, stained with methyl green in bright-field illumination.

Table 1. Nucleic acid content of *Gymnodinium nelsoni*. Nucleic acids were obtained by Schmidt Thannhauser separation. All amounts are given on an arbitrary basis corresponding to the amount in a volume of about 3 ml of packed cells.

Sample	Sugar analyses					E_{260} analyses				
	DNA		RNA		RNA/DNA	DNA		RNA		RNA/DNA
	Amt. (μg)	Recovery (%)	Amt. (μg)	Recovery (%)		Amt. (ml)	Recovery (%)	Amt. (ml)	Recovery (%)	
Intact cells	3500	(100)	3980	(100)	1.14	93.5	(100)	91.5	(100)	0.98
Brei	2840	81	2278	57	0.8	85.2	91	103	112	1.2
Purified nuclei	2940	84	346	8.7	0.118	65	69	12.9	14	0.197

light at 20°C in 10- to 15-liter volumes of enriched sea water (*2*). The cultures were harvested shortly after growth had ceased, when the cell density was about 4000 cells per milliliter. The cells were collected by semicontinuous centrifugation in a 4-liter basket centrifuge at 500 rev/min. The spent medium was sucked off from the spinning rotor. Over 80 percent of the cells from a carboy could be recovered in about 200 ml from the rim of the basket. The cells were then further concentrated in 50-ml centrifuge tubes. Since the cells disintegrate at low temperatures, it was essential to harvest at room temperature.

The harvested cells were resuspended in the isolation medium [0.25*M* sucrose, 0.005*M* $CaCl_2$, and 10 percent dextran (Pharmacia) in distilled water (weight/volume)]. All subsequent steps were carried out at 0° to 5°C. The cell suspension was passed through a Logeman mill, and the resulting brei was centrifuged at 1500 rev/min for 10 minutes. The sediment was washed twice with the isolation medium containing 0.5 percent Triton X-100 (Rohm and Haas), once with isolation medium containing 0.25 percent Triton X-100, and twice more with the isolation medium alone. After each wash, the sediment was resuspended with a Potter-Elvehjem homogenizer. The final sediment (Fig. 1b) was composed mostly of nuclei with some objects that might have been cell walls and a very few chromatophores.

The nuclei stained a brilliant blue with 2 percent methyl green in 1 percent acetic acid (weight/volume). They measured about 12 μ by 16 μ, had two broad lobes (rather like a bloated boomerang), and seemed somewhat smaller than nuclei in the living cell. Nucleoli were often visible.

The purity and integrity of the nuclear preparations were also determined by recoveries of DNA and RNA. Nucleic acids were separated by the Schmidt Thannhauser procedure (*3*) from whole cells, cell brei, and isolated nuclei (Table 1). Depending on the method of analysis and the basis of comparison, 70 to 100 percent of the DNA was recovered in the isolated nuclei. In other experiments the nuclei were found to have approximately 1.0, 1.1, and 0.16 ng of protein (*4*), DNA, and RNA, respectively, per isolated nucleus.

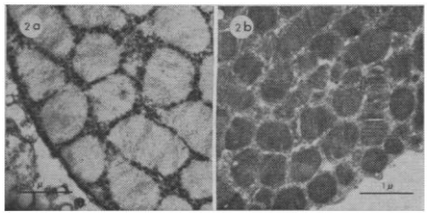

Fig. 2. (a) Electronmicrograph of nucleus in intact cell; Kellenberger fixative, Epon-Araldite embedding. (b) Electronmicrograph of isolated nucleus. Conditions for fixation and embedding were the same as those for Fig. 2a.

Table 2. Incorporation of tritiated uridine triphosphate into nucleic acid by the nuclei of *Gymnodinium*. Nuclei were obtained from a 5-liter culture of *Gymnodinium* as described in the text. The nuclei were suspended in a final volume of 5 ml containing the following: 2.5 μmole each of adenosine triphosphate, guanosine triphosphate, and cytosine triphosphate; 500 μmole of tris(hydroxymethyl)amino methane, *p*H 7.95; 5 μmole of $MnCl_2$; 20 μmole of $MgCl_2$; 50 μmole of mercaptoethanol; 500 μmole of sucrose and tritiated uridine triphosphate (Schwarz BioResearch, Inc.), 5.55×10^7 disintegrations per minute (*10*). The radioactivity of material insoluble in cold trichloroacetic acid was measured.

Time	Radioactivity in nucleic acids (count/min per milliliter)
0*	6,620
1	8,330
12	26,490
15	27,350

* Sample taken a few seconds after adding nuclei.

That the isolated nuclei retained biochemical activity was shown by the incorporation of RNA precursors (Table 2) into a fraction presumed to contain nucleic acids.

Electron photomicrograrhs (*5*) show that the peculiar chromosome-like structures characteristic of the dinophycean nucleus (*6*) were largely preserved in the isolated nuclei (Fig. 2, a and b). The nuclear membranes of the isolated nuclei appeared to be perforated; the remainder of the internal fine structure of most of the nuclei was indistinguishable from that of nuclei in intact cells.

The general method of separation was also successful with nuclei from the diatoms *Ditylum brightwelli* (West) Grun (WHOI strain DB) and *Rhizosolenia setigera* (Brightwell) (WHOI strain Rhizo) (*7*). The nuclei from the diatoms had prominent, and sometimes double nucleoli, which appeared about as large as those of *Gymnodinium*. The integrity of the nuclei of the diatoms during the separation procedure was as good as that of the dinoflagellate nuclei, but the diatoms were less suitable for detailed study. It was difficult to separate the nuclei from the clutter of broken frustules. *Rhizosolenia* grows only to low cell densities, and *Ditylum* is given to erratic and incontinent production of spores.

Dextran in the isolation medium was essential for preservation of the nuclei of all of the organisms during cell breakage. In the absence of the polymer, few intact nuclei were visible; instead, the cell brei had a stringy consistency, as if DNA or nucleoprotein had been released. Kuehl (*8*) recommends the use of a polyanionic polymer in the isolation of nuclei from higher plants. However, it was not possible to substitute dextran sulfate for dextran.

Gymnodinium nelsoni or some of its relatives which grow to denser populations may prove to be valuable "bridge" organisms between the bacteria and higher plants and animals. As judged by the cytological evidence alone, dinoflagellates are not typical eucaryotes: they lack a spindle (*9*), the structures presumed to be chromosomes remain condensed in the resting nucleus, and the peculiar "super-coiling" of these structures has been likened to corresponding forms seen in bacteria (*6*). We may imagine that, if the dinoflagellates are but one step up in nuclear complexity from the bacteria, they may

possess some of the simplest control systems elaborated in nucleated organisms.

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Gibrel: Effect on Decomposition of Plant Materials

Abstract. *Gibrel, a potassium salt of gibberellic acid, was added to various crop residues and industrial wastes, in the presence of ample nutrients, at the rate of 50 parts per million; with and without Gibrel, samples were incubated for 45 days at 35°C. Presence of Gibrel enhanced biologic decomposition of the materials; Gibrel can be used as an "activator" in composting.*

It is reported that Gibrel (*1*) affects soil respiration (*2*), numbers of *Azotobacter* (*3*), and the nitrification and sulfur oxidation of soils (*4*). We have studied its effects on microbial decomposition of several kinds of straw and of sawdust and other organic wastes that can be composted.

Eight grams of each waste material (oven-dry basis) was screened with a 10-mesh sieve and placed in a 250-ml erlenmeyer flask. The following were added: Gibrel, 50 ppm; Mo as molybolic acid and Bo as boric acid, each at 5 ppm; 1 ml of a solution of K_2HPO_4 (1 percent) and $MgSO_4 \cdot 7H_2O$ (0.3 percent); NH_4NO_3 to add 1 percent nitrogen to the organic materials (except the particle-board waste and coffee grounds, each of which already contained considerably more than 1 percent); and 1 ml of an inoculum—a suspension prepared by shaking 10 g of fresh garden soil with 200 ml of water. Water was added to bring the total moisture to 60 percent of the water-holding capacity. Control flasks were similarly treated except that Gibrel was omitted. All flasks were prepared in quadruplicate and incubated for 45 days at 35°C; water loss, determined by weighing, was replaced every 24 hours. Losses in dry matter and in carbon were used to indicate the degree of decomposition.

The plant materials were analyzed for total carbon by combustion at 1400°C, for total nitrogen by the Kjeldahl method, and for *p*H of 1:5 water suspensions by the glass electrode (Table 1). Water-holding capacity was found by saturating the material in a Gooch crucible, draining in a moist chamber, and drying at 105°C; from weight differences the water held against gravity could be calculated (Table 1).

The fact that only red-alder wood, Douglas-fir bark, particle-board waste, and spent coffee grounds were not affected by addition of Gibrel is shown by the statistical analysis of loss of dry matter and by C:N ratio. The urea-form binder in particle board is quite resistant to decomposition and requires a longer time for change in the C:N ratio to close to 10:1, which is considered a typical ratio for compost.

Maximum losses in dry matter and maximum decreases in C:N ratio were associated with the straws. Coffee grounds are relatively rich in nitrogen, and bagasse and alder wood contain more nitrogen than does coniferous sawdust (*5*). Bark is more resistant to decomposition than wood (*6*). Addition

Table 1. Analysis of waste materials before and after incubation for 45 days at 35°C.

Material	*p*H	H_2O (%)	Water-holding capac. (%)	Total carbon (%)	Nitrogen (%)	C:N ratio				Loss in dry matter (%)†	
						Before decomposition		After decomposition*			
						No N added	N added	No N added	N added	Without Gibrel	With Gibrel
					Sawdust						
Douglas fir	4.3	4.9	495	49.8	.08	622	46.1	37.2	26.1	10.4	25.7
Ponderosa pine	4.5	4.8	442	51.5	.05	1030	49.1	38.5	25.4	9.6	18.5
Hemlock	4.9	6.4	405	49.7	.04	1244	48.2	31.2	21.4	11.1	19.9
Red alder	3.2	3.9	492	49.6	.37	134	36.2	31.2	26.1	20.4	29.1
Cedar	4.4	7.1	531	51.1	.07	729	47.7	29.4	20.3	12.4	23.2
Juniper	3.9	5.8	507	48.2	.06	803	46.7	29.8	20.1	10.2	26.7
					Straw						
Wheat	5.1	5.4	460	45.1	.32	141	34.2	23.1	14.3	30.6	39.9
Oats	5.3	4.9	480	47.2	.28	168	36.9	22.4	15.1	31.9	42.3
Rye	5.8	4.6	480	47.4	.33	143	35.6	21.8	13.5	32.4	45.4
					Sundry						
Rice hulls	5.6	6.7	572	39.4	.55	72	25.3	24.2	14.9	30.4	40.1
Bagasse	6.1	6.1	648	44.9	.56	80	28.6	19.3	11.1	25.9	38.4
Douglas-fir bark	4.2	8.4	306	53.8	.16	336	46.4	41.4	38.3	9.9	17.2
Particle board	4.8	7.1	286	49.3	1.69	29	—	25.3	20.6	12.5	16.2
Coffee grounds	5.8	4.1	288	49.6	2.02	25	—	21.1	19.8	11.4	17.9

* Least significant difference at 1-percent level: C:N ratio, 8.2. † Least significant difference at 1-percent level: loss in dry weight, 6.8.