ated marrow and that this accounts for the protective effect of marrow shielding or transplants. The fact that circulating stem-type cells are not critically involved in regeneration of a small volume of depleted marrow as described here is not necessarily contradictory. For example, the probability of seeding in a small area may be quite low because of the relatively small number of circulating progenitor cells. In view of the reported abundant release of such cells from a shielded marrow, it would be of interest to study the recovery of a mechanically depopulated segment of irradiated femur when the remainder of the body has also been irradiated with exception of the contralateral femur.

Although the bone cavity appears to be devoid of marrow cells immediately after perfusion, it is, of course, possible that a few cells may be left behind. However, since stem cells represent only a small percentage of the total marrow population (*6*), the probability that any retained cells are stem cells should be correspondingly low. Mesenchymal cells are scattered throughout the Haversian system and, conceivably, some of these could serve as progenitors of the hemic colony-forming cells normally present in the marrow proper. However, the fact that donor marrow cells can more or less permanently recolonize part of a recipient's bone marrow (*7*) indicates that any influx of cells from bone is ordinarily very small, or limited to certain conditions of marrow regeneration. That cells with hematopoietic potential may be present in osseous tissue is perhaps not surprising since there are other examples of functional relations between marrow elements and bone (*8, 9*). When marrow is transplanted to an extramedullary site, the hemic cells disappear and the reticular cells that remain first give rise to bone and then to a microcirculation before hematopoietic repopulation of the implant (*8*). The picture described here is in some respects the converse since cells in bone are presumed to initiate the process of marrow repopulation. Present studies of marrow regeneration in a prelabeled femur should provide clues to the identity and location of the repopulating cells.

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Protein Content of Seed: Increase Improves Growth and Yield

Abstract. *Oat seeds with a higher protein content as a result of chemical applications in 1967 yielded 21 to 42 percent more grain in 1968. Wheat seed, whether from Michigan, Illinois, or Mexico, that contained more protein as a result of field applications of chemicals or nitrogen developed into larger seedlings. The content of protein in the seed correlated with subsequent growth and yield, indicating that the amount of endogenous protein or of a proteinaceous moiety, which can be controlled, may be an important factor in subsequent yield of major agronomic crops.*

Seed size or weight has been correlated with growth and yield; however, the explicit relation between seed protein content and subsequent growth is not known (*1*). Treatment of seeds with trace elements and fertilization of seedlings with nitrogenous compounds have increased yield, and carbohydrate and protein content of grain. Postemergence, subherbicidal rates of atrazine, simazine, and terbacil have also increased protein content of forage and grain crops (*2–4*). Wheat *Triticum aestivum* L. and oat *Avena sativa* L. seeds with higher protein content resulting from chemical treatments and nitrogen fertilization during the first generation were used to evaluate the relation of seed protein content to growth and yield in the next generation.

Total protein was determined by micro-Kjeldahl procedures (*5*), amino acids were analyzed on an automatic amino acid analyzer (*6*), and nitrate was determined (*7*). In growth chamber and greenhouse experiments, 20 uniform seeds were planted in each pot, and randomly thinned to 15 seedlings upon emergence. At harvest, plants were weighed and oven-dried for analysis (*8*).

Oat seeds from a 1967 field experiment were planted in the field on 18 April 1968, and the grain was harvested 30 July. Equal weights of seed were planted in each plot. Similar seeds were also sown in clay pots containing sandy clay loam, grown in a greenhouse at 20° to 27°C, and harvested after 19 days. Growth and yield data are the means of three replicates in randomized block designs.

The increase in seed protein content (based on Kjeldahl nitrogen) due to chemical treatment (Table 1) has been reported (*4*). However, these data show that the protein-enriched seeds as measured by amino acid analysis developed

Table 1. Relationship between seed protein (amino acids) content of oats (cv. Rodney) with growth and yield the following generation.

Chemical	Rate (kg/ha)	First generation		Second generation	
		Seed weight (mg)	Total amino acids (mg/g; dry weight)	Seedling growth (mg/plant; fresh weight)	Seed yield (kg/ha)
Control	0	29	103	187*	3830*
Simazine	.07	29	132	267	5443
Simazine	.28	30	131	282	4905
Terbacil	.14	31	127	229	4636

* F value for control compared to treatments is significant at the .05 level. The correlation of total amino acid content with seedling growth is significant at the .01 level ($r = 0.7106$) and with yield at the .05 level ($r = 0.7033$).

Fig. 1. Second generation seedlings from treated oat seed. Left to right: Control, 0.07 kg of simazine per hectare, 0.28 kg of simazine per hectare, and 0.14 kg of terbacil per hectare.

Table 2. Amino acid composition of protein hydrolysates of seed and growth of plants from seed of control and chemically treated wheat (cv. Ben Hur).*

Amino acids (mg/g dry weight)	Treatment		
	Control	Simazine (.56 kg/ha)	Atrazine (.56 kg/ha)
Aspartic acid	4.9	6.4	6.2
Threonine	2.8	4.3	4.3
Serine	4.8	6.1	7.1
Glutamic acid	30.5	43.0	44.8
Proline	7.6	10.8	12.9
Glycine	4.4	5.7	6.1
Alanine	3.6	4.5	5.1
Valine	4.5	6.0	6.8
Cystine	3.6	4.5	5.3
Methionine	1.4	1.6	2.2
Isoleucine	3.7	4.9	5.6
Leucine	6.7	9.1	9.8
Tyrosine	3.2	4.3	4.8
Phenylalanine	4.8	6.7	7.2
Lysine	3.0	3.7	3.9
Histidine	2.4	3.2	3.5
Arginine	5.1	6.6	7.4
Total	97.0	131.4	143.0
Fresh weight (mg/plant)	327	437	462
Dry weight (mg/plant)	62	83	83

* F value for treated wheat compared to control significant at the .01 level for all observations. The correlation of total amino acid content with seedling growth is significant at the .01 level [$r = 0.8610$ (fresh weight)].

into larger plants and produced more grain. Both seedling growth and yield were significantly correlated with protein content of the seed planted based on both total amino acids and Kjeldahl nitrogen. The nitrate content of the seed was not significantly correlated with growth or yield; percent germination, percent dry weight of seedlings, and seed weight was not significantly different between treatments.

Older seedlings from another greenhouse experiment differ in vigor depending on treatment (Fig. 1). Seedling emergence and development during early stages of growth are independent of treatment. After the second week of growth, plants from protein-enriched seeds were greener and had thicker stems.

Wheat seed, obtained from Michigan, with protein content enhanced by chemical treatment was grown in plastic cups containing vermiculite in a growth chamber maintained at 10°C during the night, and 20°C during the day (16 hours). A split plot design with addition or deletion of supplemental nitrogen was used. Hoagland's solution, containing 3 mM KNO_3, was added 22 days after planting. Plants not receiving nitrogen were harvested 27 days after planting and those receiving nitrogen were grown for 40 days. Seed protein (Kjeldahl N) was positively correlated with subsequent seedling growth. Seedling growth is more closely associated with protein content of seed when environmental nitrogen supply is low ($r = 0.7355$ for plants without supplemental nitrogen, $r = 0.6346$ for those with supplemental nitrogen). There was no correlation between seed nitrate and seedling growth, and percent dry weight of the forage was not altered.

Wheat seed was obtained from Southern Illinois University (*9*) and samples from three field replicates were replicated three times in the greenhouse to corroborate the preceding study. Plants were grown for 50 days, as in the greenhouse experiment. Seeds containing more protein as a result of previous chemical treatments again produced appreciably larger seedlings (Table 2). The correlation between total amino acid content and seedling growth, based on either fresh or dry weight, is significant. There was no significant difference in percent dry weight, and increases in dry weight correspond to increases in fresh weight.

Amino acid analyses of protein hydrolyzates from control and treated plants further support the relation between seed protein content as determined by micro-Kjeldahl procedures and subsequent seedling growth (Table 2).

The subherbicidal rate of chemicals used and their known metabolic fate suggest that little, if any, of these chemicals is accumulated in the seed. However, the experimental results in Table 3 establish the independence of a direct herbicidal effect in the previous tests. Higher protein content of Mexican wheat seed can only be attributed to supplemental nitrogen. Tests were conducted in the greenhouse and growth chamber as described and arranged in randomized blocks containing three replicates. Correlation of total seed protein content (Kieldahl nitrogen and amino acids) with subsequent seedling growth was significant, and percent dry weight of the forage was unchanged.

The seeds used in these tests were uniform in weight within an experiment (Tables 1 and 3). Increased seedling growth and yield in these tests are clearly not functions of seed weight. Some other substance or substances contained within the seed may have been altered by treatments used to increase protein content, and this may account for increases in growth and yield documented herein. We have established a positive correlation of seed protein and amino acid content with growth and vigor of emerging seedlings. This is logical, for degradation of storage protein, supplying amino acids necessary for *de novo* synthesis of proteins, is concomitant with seed germination.

Fortification of seed protein has immediate biological value in animal feed. It is equally important to consider the value of high protein seed in seedling growth, development, and yield. This relationship of high-protein content of seeds with seedling growth may be important to developing countries where

Table 3. Relationship between seed amino acid content of Mexican grown wheat (cv. Ciano) and subsequent seedling growth in growth chamber and greenhouse experiments.

Supplemental nitrogen (kg/ha)	Seed analysis			Growth	
	Weight (mg)	Total protein (mg/g dry weight)		Fresh weight plant* (mg)	Dry weight (%)
		Kjeldahl	Amino acids		
0	36	147	113	370 a	16.2
100	35	157	132	377 ac	16.1
200	35	161	154	415 bc	15.9
300	35	164	122	407 ab	15.5
400	34	160	152	455 d	15.5

* Means followed by unlike letters are significantly different at the .05 level (Tukey's test). F value for interaction of treatment with greenhouse and growth chamber is not significant. The correlation of Kjeldahl protein with seedling growth is sigificant at the .05 level ($r = 0.5621$). The correlation of total amino acid content with seedling growth is significant at the .01 level ($r = 0.6883$).

nitrogen deficiences may prevail and where adequate nitrogen supplies might limit future crop production. The maintenance of high protein seed for a given genotype may be imperative for the progressive development of agronomic crops.

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Actinomycin Binding to DNA: Inability of a DNA Containing Guanine To Bind Actinomycin D

Abstract. *Polydeoxy (adenylyl-thymidylyl-cytidylyl) · polydeoxy(guanylyl-adenylyl-thymidylyl), a double-stranded DNA polmer of high molecular weight containing 33 percent guanine plus cytosine, binds little or no actinomycin D, as measured by five different techniques. In contrast, the sequence isomer of this DNA, polydeoxy(thymidylyl-adenylyl-cytidylyl) · polydeoxy(guanylyl-thymidylyl-adenylyl), does bind the antibiotic. Thus, the presence of guanine in a DNA is not a sufficient requisite for the binding of actinomycin D.*

Poly d(A-T-C) · poly d(G-A-T) (*1*) was prepared by a combination of chemical and enzymatic techniques (*2*); it is a DNA polymer of high molecular weight (approximately 0.3 million daltons) containing a strictly repeating deoxyadenylyldeoxythymidylyldeoxycytidylyl sequence in one strand and a strictly repeating deoxyguanylyldeoxyadenylyldeoxythmidylyl sequence in the complementary strand. The DNA is double-stranded (*3*) and contains equal quantities of the two strands (*2*). The ability of this DNA, which contains 33 percent G+C, to bind actinomycin D was judged by spectroscopy, equilibrium dialysis, buoyant density in the analytical ultracentrifuge, absorbance-temperature transitions, and the inhibition in vitro of DNA-dependent synthesis of RNA. Little or no antibiotic is bound to this DNA under the conditions studied. This is the first example of a DNA containing guanine which does not bind actinomycin D. Thus, contrary to predictions (*4*), the presence of guanine in a DNA is not a sufficient requisite for binding.

The metabolic basis for the bacteriostatic and antitumor activities of actinomycin has been widely studied (*4*). The antibiotic binds to DNA and preferentially inhibits the DNA-dependent synthesis of RNA. The binding of actinomycin is specific for double-stranded helical DNA which contains guanine (*4–7*); single-stranded or heat-denatured DNA binds the drug only poorly, and a variety of RNA's will not bind actinomycin. In addition, a hybrid molecule consisting of the single-stranded DNA of bacteriophage alpha and its complementary RNA shows no detectable binding. Hence, it has been concluded (*4, 6*) that actinomycin binding is specific for polynucleotides containing guanine which exist in a DNA B configuration.

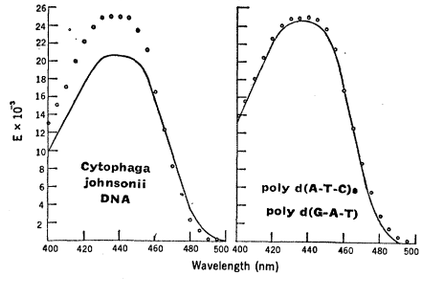

Fig. 1. Spectra of actinomycin D before and after mixing with DNA from *Cytophaga johnsonii* and poly d(A-T-C) · poly d(G-A-T). Spectra were obtained with a split compartment mixing cell (Pyrocell Manufacturing Co.) in a Cary 15 spectrophotometer. A composite spectrum was first determined with DNA (16 to 20 μM) in one compartment and actinomycin D (22 μM) in the other compartment (open circles); the cell was then inverted and mixed, and the perturbation spectra (solid lines) were determined after 10 minutes. All substances were dissolved in 0.01*M* sodium chloride and 0.001*M* sodium phosphate (*p*H 7.4).

Figure 1 shows the capacity of two DNA's, poly d(A-T-C) • poly d(G-A-T) and naturally occurring DNA from *Cytophaga johnsonii* (both containing 33 to 34 percent G+C), to perturb the visible spectrum of actinomycin D. The DNA from *C. johnsonii* induces a slight hyperchromic shift at 480 nm and a stronger hypochromic shift at 440 nm, both shifts resembling those caused by other DNA's containing guanine (*4*). Conversely, poly d(A-T-C) • poly d(G-A-T) causes no spectral perturbation outside of experimental error.

The most sensitive and thermodynamically sound technique used for detecting the binding of actinomycin to DNA is equilibrium dialysis with tritiated actinomycin. Twenty equilibrium dialysis experiments were performed on two different preparations of poly d(A-T-C) • poly d(G-A-T); the experiments were performed at 23°C in cells designed to contain as little as 0.1 ml of solution on each side of the membrane (*8*). The DNA concentration was held at $8 \times 10^{-6} M$ throughout, and the concentration of tritiated actinomycin D (Schwarz BioResearch; specific activity, 3.4 c/mmole) was varied from 0.05 to $5.0 \times 10^{-6} M$; experiments were performed in a buffer consisting of 0.01*M* sodium chloride and 0.001*M* sodium phosphate (*p*H 7.4). Equilibrium was achieved in 120 hours in the dark, with gentle shaking. The experiment was designed so that it was possible to easily detect as little as one actinomycin D molecule bound per 100 DNA nucleotides. At an actinomycin concentration ($3.2 \times 10^{-6} M$) that is saturating for a variety of DNA polymers as well as for two naturally occurring DNA's, poly d(T-A-C) • poly d(G-T-A) (*1, 2*) bound approximately 0.07 mole of actinomycin per mole of DNA phosphorus (that is, over 60,000 count/min). Under identical conditions, poly d(A-T-C) • poly d(G-A-T) showed no detectable binding of the antibiotic (less than 500 count/min). Binding isotherms for this DNA were indistinguishable from those obtained (*8*) under identical conditions for poly d(A-T) • poly d(A-T) and