of inorganic salts was markedly less than occurred in the control slices. However, if the bone slices, after exposure to saliva, were incubated in a calcifying or a strontifying medium to which glucose-1-phosphate was added, inorganic salt deposition comparable in degree to that in the control slices did occur.

It is evident that the removal of glycogen from cartilage prior to its exposure to a calcifying or strontifying solution markedly interferes with the deposition of these cations. These observations provide additional evidence of the importance of glycogen in the preparatory stages of inorganic salt deposition in cartilage *in vitro*. The influence of the addition of glucose-1-phosphate is consistent with its position in the phosphorylative glycolytic cycle.

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Plant-Growth Inhibitors from Red Kidney Bean Seeds

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In a previous publication (*1*), the writer reported the presence of photolabile germination and growth inhibitors in seeds of the red kidney bean (*Phaseolus vulgaris*). It was maintained at that time that only aqueous preparations were active and that extracts of the whole bean possessed the greatest inhibitory activity. The work reported here includes further investigation of seed coat extracts only. Somewhat different extraction techniques have yielded ether fractions containing inhibitory substances.

As before, the test object employed was the root or root and hypocotyl of the flax seedling. Flax seeds were germinated and grown in Petri dishes on filter paper; this substratum was moistened with aqueous extracts, or with water, after ether preparations had been evaporated to dryness on them. At the end of an incubation period of 45–50 hr, the length of the emerged root or of the root and hypocotyl was measured. Incubation temperature ranged from 24° to 27° C.

TABLE 1

EFFECT OF SEED COAT EXTRACTS ON THE EMERGENCE OF FLAX* (MM)

	Control	Skelly	Ether P	Ether Y	Aqueous
Length of root plus hypocotyl	10.5	10.8	3.9	7.8	6.0
Percentage of control	100	103	37	74	57
Density at 320 mμ	....	....	0.600	0.600	...
Density at 585 mμ	....	....	0.179	0.055	...

* Based on 30–50 seedlings incubated 48 hr with 3 ml of extract; extracts in organic solvents were dried on the filter paper, and the 3 ml of water then added.

Whereas in previous work attempts to extract dry seed coats with ether failed to disclose inhibitors, extraction with ether in the presence of water yielded clear, purple ether fractions with activity. Extracts were obtained as follows: Bean seeds were soaked in water at room temperature for no more than 3–4 hr to soften seed coats; these were then removed. For extract P-3, 10 g of dry seed coats, 30 ml of water, and 30 ml of diethyl ether were placed in 125-ml Erlenmeyer flasks and kept in darkness, with occasional shaking, for about 12 hr. The ether fraction, by this time a deep, clear purple, was separated, shaken 2–4 times with the red-orange aqueous phase, and dried over anhydrous sodium sulfate. Extracts were stored in darkness until use. All operations were conducted in darkness or in diffuse light that did not exceed 15 ft-c intensity. Extract P-4 was prepared using 10 ml of water, 50 ml of ether, and an 8-hr extraction period; continuous agitation was maintained, using a reciprocal shaker. By this method, 100 g of seed coat was extracted, and the ether fractions were concentrated *in vacuo*; the concentrate was dried at 5° C over sulfuric acid.

Other modifications of the techniques described have been successfully employed. Using a Beckmann Model DU spectrophotometer, absorption spectra of the ether extracts were studied for several reasons: (1) once having established the characteristic absorption for the first extract prepared, the presence of the same absorption peaks (Table 1) in subsequent preparations served as a check on the extraction method; (2) it was desirable to find if destruction of the purple pigment in the extract (through the agencies of light, alkali, etc.) was correlated with changes in the inhibitory properties of the preparation.

The relative activities of several preparations are given in Table 1. The greatest activity was found in the purple ether fraction (Ether P); shaking this preparation with sodium hydroxide pellets transformed the extract to a yellow color and diminished its activity by a factor of two (Ether Y).

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Exposure of purple ether fractions to white or yellow light of 1,000 ft-c intensity for periods of 0–30 min resulted in a decrease in absorption at 585 mμ from 0.179 at 0 min irradiation to 0.075 at 30 min; there was no significant decrease in inhibitory activity. Irradiation for 45–50 min, on the other hand, reduced the absorption to 0.015 and the inhibitory activity from 45% of control to 60–63% of control.

The concentrate of extract P-4 yielded 40 mg of a red-brown solid from 100 g of seed coat. This solid was no longer soluble in ether to any extent and failed to give any purple coloration. Further, the inhibiting activity was one half as great as that of freshly prepared ether solutions. This concentrate was completely inactivated by 60 min exposure to 200–400 ft-c of white light, when in aqueous solution.

In another set of experiments, ether fractions were applied to carefully weighed disks of 9-cm filter paper. These were weighed again after the ether had been evaporated. Quantities of this concentrate ranging from 16 to 100 mg of dry material were deposited on the filter papers (the error in this method is 5–10%). Applying 2–4 ml of water to these disks in Petri dishes, concentrations of 4–50 mg/ml were obtained. Flax seeds were then germinated on the filter paper, and measurements of root length taken after 45 hr incubation. With concentrations below 20 mg/ml, irregular results were obtained, but using concentrations from 20 to 50 mg/ml, regular results were obtained. At 0 mg/ml, root growth in the stated interval was 4.4 mm; with 20 mg/ml, 4.2 mm; with 30 mg/ml, 3.3 mm; with 50 mg/ml, about one third of control growth was obtained. Irradiating the extract having a concentration of 30 mg/ml with 500 ft-c of white light for 15 min completely inactivated the inhibitor. Figures are based on 30–35 roots per group.

These inhibiting solutions were also observed to suppress root hair development considerably.

There is some indication that the ether-soluble system is thermolabile. Previously, the aqueous seed coat extract was found to be inactivated by heat (*1*).

Although Barton and Solt (*2*) reported inhibitory activity in the seed coats of pole bean varieties (*Phaseolus* sp.), they found greater activity in aqueous extracts than in those made with organic solvents, as did the present author previously. It is evident from Table 1 that the ether extracts have higher activity than aqueous preparations; whether this is a result of differences in concentration or in the nature of the inhibitor is not known.

The evidence presented here indicates that a relationship may exist between the purple coloration of the ether fractions and at least some of the inhibitory activity of these extracts. Both light and alkali diminished biological activity and destroyed the pigment. This indicates either the presence of two or more inhibitors or of a single labile inhibitor molecule. Among the effects of cold alkali on organic molecules is its ability to open lactone rings; further, some lactones are known to exert effects on plant growth (*3*, *4*). Finally, it is of interest to note that like auxins *a* and *b*, the ether fraction of the bean seed coat is sensitive to alkali (*3*, *4*). These latter observations are of a speculative nature, but may give some clue as to the kind of substance or substances acting as inhibitors.

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Adjuvant Action of Amino Acids and Peptides in Fertilizin Agglutination of Starfish Sperm[1]

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Starfish (*Patiria miniata*) sperm in 1–2% sea-water suspension are generally immobile and do not agglutinate appreciably upon addition of the specific isoagglutinin fertilizin. However, when treated with an appropriate adjuvant (hen's egg white or various vertebrate or invertebrate sera) the sperms become intensely active, agglutinate strikingly and specifically upon addition of homologous fertilizin, and show a marked increase in fertilizing power (*1*).

In an attempt to discover the chemical nature of the adjuvant, a series of amino acids and related substances was tested for adjuvant action on the Pacific webbed star, *P. miniata*. Most of the α-amino acids and peptides proved to be very effective adjuvants. All substances tested were prepared as 0.1*M* solutions (saturated solutions in the case of less soluble compounds) and the pH of each solution was adjusted to that of sea water (pH 7.9) with 1N HCl or NaOH. In most of the tests 1 vol of 1–2% *P. miniata* sperm and 2 vol of test solution were mixed, and finally 1 vol of fertilizin was added. Controls for the action of sea water, test solution, and fertilizin alone were run in all cases. An experiment was rejected if agglutination occurred in any of these controls.

The adjuvant action of amino acids and peptides appears to be identical with that of hen's egg white and sera, previously described (*1*). Thus the agglutination resulting from addition of fertilizin to amino acid- or peptide-treated *Patiria* sperm is exclusively head to head and does not reverse within a limited time. Furthermore, the reactions are species-specific. Specific agglutination reactions but no cross-reactions were observed with amino

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