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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TABLE 1

ADJUVANT ACTION OF AMINO ACIDS AND RELATED SUBSTANCES IN FERTILIZIN AGGLUTINATION OF *Patiria miniata* SPERM

	Substance tested	Adjuvant action or adjuvant titer*	
l	Cystein	4,096	
	Glutathione	512–1,024	
l	Proline	64	(all reactions very weak)
dl	Leucine†	64	
dl	Alanine	32	
dl	Valine	32	
	Glycine	16	
l	Lysine	4–8	
d	Lysine	4	
	Glycylglycine	2	
	Glycine methyl ester	2	
dl	Glutamic acid†	2	
dl	Phenylalanine†	+	
l	Aspartic acid†	+	
l	Tryptophane	+	
	α-amino butyric acid	+	
	β-amino butyric acid	−	
	β-alanine	−	
	N-methyl glycine	+	(all reactions very weak)
	N-dimethyl glycine	−	
	Hippuric acid†	−	
dl	Acetyl valine	−	
	Acetate	−	
	Pyruvate	−	
	Acetamide	−	
	Ethyl amine	−	
	Ammonium chloride	±	

* Reciprocal of highest dilution of 0.1*M* solution showing adjuvant action. All titrations were not run simultaneously. Titers are related to glycine taken as 16.

† Saturated solution.

acid-treated sperm and fertilizin of *P. miniata*, *Astrometris sertulifera*, and *Pisaster ochraceus*.

Examination of Table 1 shows that every one of the α-amino acids tested showed adjuvant action. This clearly indicates that at least within wide limits the α-amino acid structure is effective regardless of the attached radical. However, the activity of the various amino acids and peptides is not independent of the attached residue, as can be seen by comparing the adjuvant titers shown in the table. Both the D and L forms of the amino acids seem to be effective, since both isomers of lysine were active. The α structure is not only effective but would seem to be required, since the β-amino analogues of alanine and amino butyric acid produced no adjuvant effect.

Apparently esterification or peptidization of the carboxyl group, as in glycine methyl ester or glycylglycine, respectively, reduces but does not destroy the activity of an amino acid. However, a free α-amino group would appear to be essential for satisfactory adjuvant activity. Thus the α-imino acids proline, sarcosine (N-methyl glycine), acetyl valine, and hippuric acid showed little or no activity and the tertiary amino derivative N-dimethyl glycine was completely inactive. In view of these considerations, it would appear that a free amino group and a carboxyl (free or esterified, but preferably free) group on adjacent carbon atoms is one essential structure for adjuvant action.

A carbonyl group in place of a carboxyl group, as in the peptides, may be sufficient for adjuvant action. However, it is possible that peptidases hydrolyze the peptides to amino acids and that the latter are responsible for the adjuvant action of peptides. Assuming that a carbonyl group can substitute for a carboxyl group, the essential structure outlined above can account for the adjuvant action of crystalline ovalbumen (1% sol) and crystalline bovine serum albumen preparations observed in this study. Assuming that peptide linkages are broken or that masked terminal α-amino groups of proteins are exposed by boiling or by ultraviolet irradiation, the increase in adjuvant action of egg white and the presence of a heat-stable, nondialyzable (polypeptide?) adjuvant(s) in egg white and sera (*1*) are explained. The heat-stable egg white proteins lysozyme and ovomucoid (trypsin inhibitor) are inactive. Finally, the assumption that an increase in α-amino groups occurs through bacterial hydrolysis of protein explains why aged *Patiria* fertilizin solutions stimulate and agglutinate homologous sperm in the absence of added adjuvant (*2*).

The mechanism of adjuvant action of the amino acids has not been investigated, but presumably the amino acids operate in the same manner as the poorly defined adjuvant of hen's egg white. The available evidence (*1*) indicates that the latter acts upon the sperm to expose more antifertilizin groups on the sperm surface, thereby converting the normally "univalent" sperm to a multivalent agglutinable form. How the amino acids could achieve such a conversion is not clear. The effective chemical structure outlined above, and the fact that the active agents stimulate the sperm to intense activity, suggest that sperm metabolism is involved in the adjuvant effect. However, the immediate products of amino acid oxidase action are not effective adjuvants. Thus pyruvic acid, the α-keto analogue of alanine, proved to be completely inactive, and ammonia showed only a very slight adjuvant effect at one concentration in one experiment. Since alkali has a very striking adjuvant effect on starfish sperm (*2*), it is tempting to ascribe the adjuvant effect of amino acids to an increase in pH as the result of the release of ammonia. However, addition of alanine produced no detectable pH change in an *Asterias forbesii* sperm suspension. Furthermore, a decrease rather than an increase in pH should be the immediate result of amino acid oxidase action on alanine, since pyruvic acid is more dissociable than ammonium hydroxide (*3*). Nevertheless, if the keto acid were metabolized to a less dissociable acid (such as CO_2 via the citric acid cycle), leaving the ammonia to accumulate, a local if not an over-all rise in pH could result even in a highly buffered solution. Whether this is the actual mechanism of action of the amino acids can only be decided by further study.

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