

during the period of the experiment is of considerable interest.

To test the effect of DL-methionine sulfoximine on the uptake of  $S^{35}$ -labeled DL-methionine by liver slices, they were washed in cold Krebs' isotonic medium for 30 min, blotted dry, and weighed. Approximately 0.5-g samples were incubated in 15 ml Krebs' isotonic solution at pH 7.4 for 130 min at 37° C in an air atmosphere. The media contained (1) 30 mg  $S^{35}$  DL-methionine/100 ml, (2) 30 mg  $S^{35}$  DL-methionine, plus 30 mg of unlabeled DL-methionine sulfoximine/100 ml. Activity of the methionine was 264,000 cpm/mg as counted in a "Q" gas tube. The results are given in Table 1.

The low uptake of methionine sulfoximine by brain and spinal cord indicates the probable absence of a direct effect of the drug on these tissues. One possible explanation for its action suggested by the results in Table 1 could be through its inhibition of methionine uptake by nerve tissue and thus inhibition of the synthesis of enzymes important to nerve metabolism. Additional work is under way to test this and other possibilities.

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## Growth-Regulator Specificity in Relation to Ovary Wall Development in the Fig

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Calimyrna figs produced parthenocarpically by growth-regulator application are seedless (1-3). In addition to the fact that embryo development does not take place, the endocarp, or inner ovary wall, of the fruitlets (achenes) does not sclerify and impart hardness to the so-called "seed" as in the case of pollinated syconia (4). Parthenocarpic Calimyrna fig syconia produced during the 1951 season with benzothiazol-2-oxyacetic acid,<sup>1</sup> however, have been found to contain achenes with the endocarp tissue as completely sclerified as in syconia resulting from cross-pollination. Thus, parthenocarpic figs produced with this compound have a texture identical to those produced

by the usual commercial procedure of caprification (cross-pollination).

These results demonstrate the high degree of specificity of the growth-regulating substances and the diverse reactions they induce. During the past five years, several growth-regulators have been found to induce parthenocarp in the Calimyrna fig, a variety that requires the stimuli of pollination and fertilization in order for the syconia to set and mature. Ovary wall development of achenes in syconia induced to develop parthenocarpically by three of these compounds has been investigated histologically. In each case some pronounced modification from the normal development and composition of the ovary wall has been revealed. In syconia produced with  $\gamma$ -(indole-3)-*n*-butyric acid, the endocarp in the individual achenes was completely absent (4). The endocarp of achenes in syconia produced with *p*-chlorophenoxyacetic acid, on the other hand, was observed to have developed, but typical sclerification of this tissue did not take place. Hence, the tissue did not become hard, but remained parenchymatous in character. Figs produced with this compound have become known commercially to the trade as "miracle seedless figs," because they do not contain achenes which characterize pollinated figs.

Benzothiazol-2-oxyacetic acid, when applied at a concentration of 100 ppm, induced 100% of the unpollinated but pollen-receptive syconia to set and mature parthenocarpically, with no injury to fruits or foliage. Of these, 80% matured at the same time as pollinated syconia, and 20% of the induced parthenocarpic figs reached maturity 3 weeks following the date of spray application, a response similar to that obtained with 2,4,5-trichlorophenoxyacetic acid (5). The syconia that matured approximately 6 weeks previous to the time of maturity of pollinated syconia contained achenes, the endocarp tissue of which did not sclerify. The process of endocarp sclerification did not take place, apparently because the time interval between growth-regulator application and maturity of the syconia was too short. Benzothiazol-2-oxyacetic acid-induced parthenocarpic syconia that matured at the same time as pollinated syconia, however, contained achenes with a completely sclerified endocarp. With the exception of slight differences in color of the pulp and lack of both endosperm and embryo development in the achenes, the parthenocarpic syconia appeared identical to pollinated syconia as regards shape, size, and texture.

It is significant that the most pronounced influence of the growth-regulating compounds inducing parthenocarp in the Calimyrna variety of fig is morphologically manifest in a discrete tissue of the ovary wall. Hence, depending upon the growth-regulator used to induce parthenocarp, distinctly different achenes can be produced. The following three distinct modifications from normally pollinated syconia, for example, are now possible in the Calimyrna fig: (a) syconia containing achenes in which the endocarp is lacking, [produced with  $\gamma$ -(indole-3)-*n*-butyric acid]; (b) syconia containing achenes in which the

<sup>1</sup> Supplied by the American Cyanamid Company.

endocarp is present but not sclerified (produced with *p*-chlorophenoxyacetic acid); and (c) syconia containing achenes in which the endocarp is present and completely sclerified (produced with benzothiazol-2-oxyacetic acid). Achenes in each of these types do not contain an embryo.

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## Protective Effect of Glycine on Sperm Exposed to Light

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Spermatozoa of the purple sea urchin, *Strongylocentrotus purpuratus*,<sup>2</sup> were found to be about a million times as sensitive as eggs to ultraviolet light (1) at wavelengths absorbed by nucleoproteins. Spermatozoa of a number of other sea urchins, *S. franciscanus*, *Arbacia punctulata*, the sand dollar *Dendraster eccentricus*, and the starfish *Pateria miniata* were also found to be highly sensitive to these radiations (2). The injurious effect of ultraviolet light on *S. purpuratus* is partially reversed by small dosages of monochromatic visible light, especially at 4350 Å (3). However, visible light also has an injurious effect on spermatozoa, immotilizing them and rendering them incapable of fertilizing the eggs. Dosages that do not immotilize the spermatozoa cause a slight delay in cleavage (3). The recent discovery by Tyler (4-6) that glycine protects spermatozoa against deterioration after dilution of the spawn suggested the possibility of protecting the spermatozoa against radiations by this means.

Accordingly, in all the following experiments on this species, the spawn issuing from a testis of *S. purpuratus* was diluted 1:400 in sea water solution containing 0.05M glycine (this being the concentration found optimal by Tyler), a similar suspension in sea water alone serving as control. A drop (about 0.01 ml) was withdrawn from each vessel at various periods and tested by adding to a dish containing about 300 eggs in 0.3 ml sea water, which was stirred by gently blowing with a pipette. Although there is variation in the longevity of sperm from different animals, untreated but diluted spermatozoa kept at 13° C in the dark lose their viability in 1-3 hr. In diffuse daylight

(100-200 ft-c) and at a higher temperature (21° C), they remain virile less than an hour. In the presence of glycine, however, they remained virile for more than 20 hr (the longest time tested) in the dark at 13° C. Spermatozoa of the purple sea urchin are therefore protected by glycine in the same manner as those of the other species tested by Tyler and co-workers.

Glycine, however, offers no protection against injury by short ultraviolet radiations. Spermatozoa without as well as with glycine, irradiated with 50 ergs/mm<sup>2</sup> of λ 2654Å from a quartz monochromator, were used to fertilize normal eggs. The resulting zygotes in both cases showed an 80-min delay in the first cleavage over unirradiated controls (for methods see [3]). Nor did the presence of glycine enhance photoreactivation by visible light of spermatozoa injured by ultraviolet radiations, the cleavage delay being the same in experimental and control.

On the other hand, glycine had a strong protective effect on spermatozoa treated with visible light. In most of these experiments the source of radiations was a GE CH-4 spotlight (at 30 cm), and the light was filtered through 8 in. of water and a #3060 Corning glass filter, which cuts off most of the long ultraviolet (λ 3660Å and possibly somewhat shorter) but transmits most of the visible (30% at 4000Å). The intensity, measured by a GE exposure meter, was about 3900 ft-c. Spermatozoa in sea water exposed to this light lost their viability in 5-10 min. In glycine solution they fail to fertilize eggs only after exposures of about an hour or more. Addition of glycine to spermatozoa that have been immotilized with white light does not revive them, whether the suspension had been treated in sea water or in glycine sea water. Treated with sunlight (1800 ft-c) through water cells to remove heat and the Corning #3060 filter to remove the ultraviolet radiations, the spermatozoa were killed in 10 min in sea water, but those in glycine sea water were still fully viable after 33 min.

Of the wavelengths tested, the visible light that is most effective in immotilizing spermatozoa of the purple sea urchin is the blue, λ 4350Å; a dosage of 20,000-40,000 ergs/mm<sup>2</sup> affects almost 100% of the spermatozoa, as judged both by immotilization and by the failure to induce fertilization membranes or cleavage (the latter check being necessary since occasionally one finds membranes so close to the egg that fertilization might be questioned, yet cleavage occurs). Violet light, λ 4050Å, is less effective than blue, immotilizing only after dosages of 60,000-70,000 ergs/mm<sup>2</sup>. Wavelength 3660Å in the ultraviolet was not effective even at dosages of 80,000 ergs/mm<sup>2</sup>, the highest tried. Monochromatic yellow (5780Å) was also ineffective even at a dosage of 80,000 ergs/mm<sup>2</sup>. The compound responsible for the injury therefore seems to be something which absorbs strongly in the blue-violet portion of the visible.

The eggs of the purple sea urchin, although quite resistant to visible light filtered through the Corning #3060 filter, are also sensitive to the total radiations

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<sup>2</sup> Collected at Moss Beach, Calif., for the work at Stanford and off the rocks near the laboratory for the work at the Hopkins Marine Station.