Technical Papers

Effect of Phosphorylated Hesperidin, a Hyaluronidase Inhibitor, on Fertility in the Rat

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Four experimental observations led to the currently recorded experiments. McClean and Rowlands (*1*) and Fekete and Duran-Reynals (*2*) have reported that the ovum in the fallopian tube is covered with follicular cells embedded in a thick viscous gel, chemically consisting primarily of hyaluronic acid (*3*). The sperm cannot penetrate the ovum unless the outer hyaluronic acid gel layer is dispersed. McClean and Rowlands (*1*) found that testicular hyaluronidase added to recently ovulated ova causes a dispersion of the follicular cells, denuding the ova and thus permitting sperm penetration. Clearly, the mechanism is dependent upon the activity of hyaluronidase, and a highly effective inhibitor of hyaluronidase should block fertility. *In vitro* and *in vivo* studies (*5*) have demonstrated the capacity of hyaluronidase inhibitors to prevent the follicle-cell-dispersing activity of rabbit sperm hyaluronidase and to act as contraceptives. Phosphorylated hesperidin (*4*) is a powerful inhibitor of hyaluronidase and is nontoxic. We therefore undertook to determine the capacity of phosphorylated hesperidin as a hyaluronidase inhibitor to produce sterility.

The fertility experiments were done with rats, in groups of three, each cage containing one male, one control female, and one female being given phosphorylated hesperidin. In cages where the control females did not deliver, the results were not included in the final tabulation. This served as a check on the fertility of the males used. Females were examined periodically for vaginal plugs to insure that coitus had taken place. The phosphorylated hesperidin was given either intraperitoneally at 20 mg/kg or orally at 100 mg/kg. In each case the controls were treated with equal volumes of saline or water.

The experiments were continued until two weeks after the last control delivery. At this time the animals were separated and treatment with phosphorylated hesperidin was stopped. The animals were kept under observation for a further three-week period, and were then remated with the males, this time without being given phosphorylated hesperidin.

Phosphorylated hesperidin was found to be an effective antifertility agent, whether administered intraperitoneally or orally. In the group receiving the compound orally, 6 rats out of 30, or 20%, became pregnant and had successful deliveries. In the group being given intraperitoneal injections, 4 rats out of 24 rats, or 17%, delivered. Thus, of a total of 54 rats, conception was prevented in 44. Since the cages in which the control rats did not conceive were discarded, the control rate has been 100%. In no cage where a control rat failed to conceive did an experimental animal become pregnant.

Approximately 80% of the experimental animals became pregnant when they were remated after treatment with the phosphorylated hesperidin was stopped. The material when administered only to the males had no effect; the action appears to be specifically on the female. Examination of vaginal smears showed that the estrus cycle remains normal during the administration of phosphorylated hesperidin.

The results reported here, although striking, must be considered to be merely preliminary, because of the small number of animals used. Further work, with much larger numbers of animals, is now in progress.

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Uracil Protection against Ultraviolet Radiation Damage to a Higher Plant

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It is known that sublethal ultraviolet irradiation of bacteria and plants damages the cell division mechanism and inhibits growth (*1, 2*).

Limperos and Mosher (*3*) have shown that thiourea will reduce the mortality rate of x-irradiated mice. They have theorized that this reduction of mortality may be due to protection of the nucleic acids of the cell by thiourea. One of the basic constituents of the nucleic acids of the cell is uracil. That pyrimidine derivatives are destroyed by ultraviolet light has been shown by Rapport and Canzanelli (*4*). Sinsheimer and Hastings (*5*) have likewise found that irradiation with ultraviolet light for 15 hr at a pH of 7.0 caused a 63% loss in the maximum absorption of uracil. Puleston (*6*) has shown that bacteria, treated with a lethal dose of ultraviolet light, can be both reactivated and protected from the effects of this radiation by uracil.

The present paper is concerned with the measure-

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TABLE 1

THE PROTECTIVE EFFECT OF URACIL AGAINST ULTRAVIOLET RADIATION (2537 A)* DAMAGE ON ALASKA PEA SEEDLINGS

Variance analyses						
Variability due to	D/F†	Sums of squares	Mean squares	Observed F	Required F .05	.01
1. Control (sprayed with distilled water and irradiated) —Treatment (500 μg uracil)						
Totals	23	6.098				
Between treatments	1	1.402	1.402	6.56	4.30	7.94
Within treatments	22	4.696	0.213			
Standard deviation = 0.46 Standard error = 0.42						
Mean growth for control = 2.41 cm; and for treated = 2.93 cm, with a difference of 0.52 cm						
Minimum difference required for significance at .05 level, 0.39; at .01 level, 0.53						
2. Control (sprayed with distilled water and irradiated) —Treatment (1000 μg uracil)						
Totals	23	11.311				
Between treatments	1	3.700	3.700	10.70	4.30	7.94
Within treatments	22	7.611	0.346			
Standard deviation = 0.59 Standard error = 0.17						
Mean growth for control = 2.41 cm; and for treated = 3.23 cm, with a difference of 0.82 cm						
Minimum difference required for significance at .05 level, 0.50; at .01 level, 0.68						

* A 30-w GE germicidal tube No. G-30, T-8 (mercury arc) at a distance of 20 cm. This tube operates on the principle of the fluorescent tube, but the glass of the tube is clear and of a special type which passes a particular wavelength of ultraviolet. About 95% of this ultraviolet energy is in the region of 2537 A—the most effective germicidal energy.

† D/F = degrees of freedom.

ment of the effect of uracil in protecting 6-day-old etiolated Alaska pea seedlings against ultraviolet radiation (2537 A, Table 1).

Seedlings were sprayed with solutions of uracil and irradiated for 30 min. Approximately 25 ml of spray was used on each lot of 60 seedlings. Each lot consisted of 12 bands containing 5 seedlings each, making a total of 60 plants per lot. Three types of controls were used. One was sprayed with distilled water and irradiated, one not sprayed but irradiated, and one sprayed with 1000 μg of uracil but not irradiated. Details are given in Table 1 and in the following discussion. Height measurements before and 48 hr after treatment were used as a measure of the effectiveness of uracil as a protector against ultraviolet radiation damage.

The variance analyses show that 500 μg of uracil sprayed on pea seedlings significantly protected them from ultraviolet radiation damage at the 5% level and that 1000 μg of uracil significantly protected them at the 1% level. Uracil at the rate of 200 μg gave no significant protection.

When comparisons were made between the following two control lots, one receiving no uracil, no irradiation, and no water spray, the other receiving 1000 μg of uracil spray but no irradiation, differences were not significant. This demonstrated the lack of phytotoxicity of uracil at this level.

These results are in accordance with the results previously observed by Puleston on *Streptococcus faecalis* R. The present work suggests that uracil has a similar protective action for a higher plant.

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Metabolic Relationship between *Meso*-Inositol and Lindane

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A close relationship of the spatial configuration of *meso*-inositol and lindane (γ-benzene hexachloride) molecules was reported (*1*) in 1945, and following this observation workers (*2–8*) have investigated the mechanism of this interrelationship on a variety of organisms. In insects (*9–10*), however, no inhibition of the γ-isomer by *meso*- or *m*-inositol has been found after the simultaneous administration of these compounds.

The purpose of this note is to determine whether there exists any metabolic relationship between *meso*-inositol and lindane in the German cockroach, *Blattella germanica* Linn. For this investigation one female roach carrying the egg pod was placed in each of 16 battery jars containing roach food (*11*) (10 parts whole wheat flour, 9 parts dried skim milk powder, and 1 part dry baker's yeast) mixed with *meso*-inositol in percentages of 0, 0.5, 1–10, 15, and 25. The hatching date of each pod was noted, and the nymphs were allowed to feed in the battery jars for 38 days.

A group of 10 roaches bred on each concentration of inositol was placed in three Petri dishes separately containing the same roach food (*11*), mixed with 0.1% γ-, 10% α-, and 10% β-isomers of benzene hexachloride, respectively. Each dish contained a cir-

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