of arginase activity in malignant tissue (*2*); possibly this activator is sex-linked.

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Further Study of the Role of Hyaluronidase in the Fertilization of Rabbit Ova *in Vivo*[1]

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In a previous experiment (*4*), superovulated doe rabbits were inseminated with 0.5 ml of a sperm suspension (1: 1,000 in 0.9% NaCl) added to 0.5 ml of 0.9% NaCl containing partially purified testis hyaluronidase. Although the number of fertilized ova was not increased by the addition of hyaluronidase, it was thought that the dilution effect (*3, 6*) may have reduced fertilizing capacity and that the partially purified hyaluronidase may have contained inhibitory impurities. Since hyaluronidase is thought to be of therapeutic value in oligospermia in human infertility (*8*), a further test of a possible hyaluronidase effect on the fertilization of rabbit ova *in vivo* was conducted.

Seminal plasma has been demonstrated to be beneficial to the fertilizing capacity (*4, 5*) and to the motility (*6*) of spermatozoa in the highly diluted form. Therefore, 0.003–0.01 ml of rabbit semen was suspended in a fluid containing 1 ml of vasectomized seminal plasma (which itself has no hyaluronidase activity) and 9 ml of Fructose Ringer's solution (*5*). In one group of animals, 1 mg of highly purified testis hyaluronidase[2] in 0.5 ml of Fructose Ringer's solution was placed into the upper part of the vagina. The animals were then inseminated with 1 ml of the sperm suspension. In the second group, rabbits were inseminated with 1 ml of the sperm suspension containing 1 mg of purified hyaluronidase. In a third group, the animals were inseminated with 1 ml of the same sperm suspension containing 1 mg of lyophilized, vasectomized rabbit seminal plasma. The number of spermatozoa in the sperm suspension was estimated with a hemocytometer. The animals were injected intravenously with sheep pituitary extract just after insemination in order to induce ovulation. They were sacrificed 25 or 72 hr later. The ova were recovered by flushing the tubes, and fertilized as well as unfertilized ova were counted.

Results are presented in Table 1. The average percentage of fertilized ova is 50 and 59 in the experimental groups, and 46 in the control group. The percentage of total fertilized ova is 42 and 55 in the experimental groups, and 39 in the control group. There is no significant difference between each group, either calculated according to the χ^2 test or according to the t test. It is not established, therefore, that the application of hyaluronidase in the vagina before insemination or the addition of hyaluronidase to the sperm suspension has any effect on the fertilization of rabbit ova *in vivo.*

TABLE 1

EFFECT OF PURIFIED TESTIS HYALURONIDASE ON FERTILIZATION *in Vivo*

Experiment No.	No. of sperm inseminated	No. of ova*		% fertilized	No. of ova†		% fertilized	No. of ova‡		% fertilized
		Fertilized	Total		Fertilized	Total		Fertilized	Total	
1	250,000	8	34	24	22	26	85	9	32	28
2	210,000	7	15	47	10	10	100	21	22	96
					8	28	29			
3	150,000	24	27	89	7	10	70	15	17	88
4	50,000	8	12	67	38	55	69	8	47	17
5	80,000	2	10	20	5	11	46	6	22	27
6	93,000	1	58	2	4	30	13	2	41	5
7	300,000	27	27	100				29	50	58
Avg. % fertilized ova per doe				50			59			46
Total ova in each group		77	183	42	94	170	55	90	231	39

* Hyaluronidase (1 mg) placed into vagina before insemination.

† Hyaluronidase (1 mg) added to sperm suspension.

‡ Dried vasectomized semen (1 mg) added to sperm suspension.

The hyaluronidase used in the present experiment was tested by the viscosimetric method (*7*). It was estimated to be about 200 times as potent as the partially purified hyaluronidase used in a previous experiment (*4*). Vasectomized semen has no hyaluronidase activity

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[2] Kindly supplied by Dr. Joseph Seifter, and containing 800 turbidometric units per mg.

(*4*), and the hyaluronidase activity of the sperm suspension employed in the present control group was practically nil. However, the percentage of fertilized ova in the control group and in the experimental group was very similar. The motility of spermatozoa in the sperm suspension with or without hyaluronidase was the same; all showed active motility in the highly diluted form at room temperature for 6 hr. The role of hyaluronidase as determined by the observation of dispersing cumulus cells *in vitro* does not appear to be so important in fertilization as previous investigators have thought because fertilized ova were still observed in cumulus cells (*1*, *2*, *9*). The experimental findings here reported fail to support the claims made for the clinical use of hyaluronidase.

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A Simple Staining Technique for Detecting Virus Diseases in Some Woody Plants[1]

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During the course of biochemical studies on virus diseases affecting deciduous fruit trees, it was observed that virus infection induces the accumulation of phenolic compounds in affected tissues. A color test for these compounds was developed (*2*), which proved useful in the study of various stone-fruit virus diseases.[2] Further work suggested that if these phenolic compounds could be fixed in the tissue and subsequently stained, they might serve as a means of observing virus distribution in the plant. Such a test was devised for the staining of phenolic compounds in cleared leaf tissue. The stained compounds are not distributed uniformly throughout the tissue but are located in patterns which are characteristic for the type of virus and for the severity of infection. The technique is useful in detecting and studying what appear to be virus movement and distribution in plants capable of producing polyphenols similar to those produced by deciduous fruit trees.

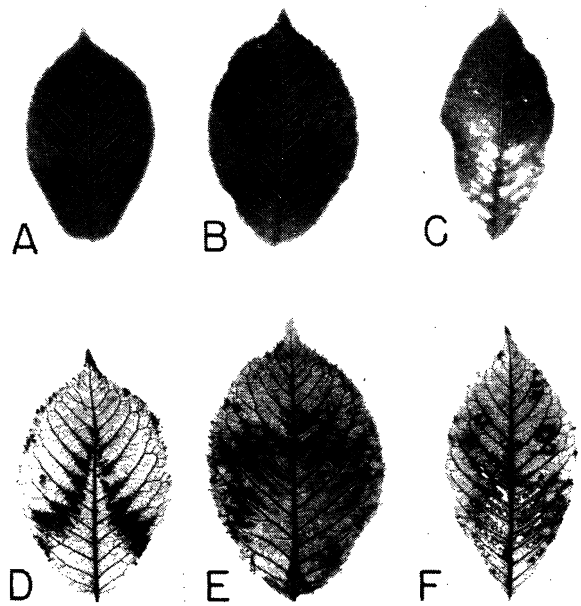

FIG. 1. Bing cherry leaves affected with ringspot. A, B, and C, before clearing and staining; D, E, and F, the same leaves after staining. The shot holes in C and F are caused by the virus.

The staining procedure consists of removing the chlorophyll, fixing the polyphenols, and developing blue-colored polyphenol compounds by treatment with NaOH. Whole leaves or sections of leaves were used. This procedure was also used with sections of petioles, stems, and roots. The decolorizing and fixing are performed in one operation by using a reagent composed of 700 ml of 95% ethyl alcohol, 20 ml of 37% formaldehyde, and 230 ml of distilled water. Samples may either be boiled in this solution under a condenser, or they may be heated in a water bath at 80° C until the chlorophyll is completely removed. When the clearing and fixing reagent becomes strongly colored, it should be discarded and fresh reagent added. One or two changes are usually sufficient. The chlorophyll from young leaves is removed in 5 to 10 min, but older leaves may take as long as 2 hr to clear. When decolorized, the leaves appear white or light yellow.

After decolorization, the samples are transferred to normal NaOH and heated at 80° C to 100° C until maximum color develops. The time of heating varies with the type and age of the sample but is usually from 2 to 10 min. A deep blue color indicates a test reaction between polyphenols and NaOH. Tissues in which these phenolic compounds are absent are yellow. The blue color of the phenolic compounds stands out sharply

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[2] A recent comment in *Science* (*1*), suggesting that our original color test for virus diseases was due to reducing substances, is in error. A small amount of copper sulfate was added to the reagent for the colorimetric test, to catalyze the oxidation of the polyphenols. The author of the comment assumed that a cuprous oxide precipitate was the basis for the test, whereas a red-colored solution was originally reported. Copper is not essential to color formation in this test.