verted into fermentable sugars if they are continuously removed by yeast fermentation.

The data reported here confirm the report by Pigman (*6*) that a mold enzyme or enzymes can convert maltose to some unfermentable carbohydrate or carbohydrates. The possible occurrence of such enzyme or enzymes in amylolytic preparations undoubtedly explains the apparent inhibition of amylase activity by maltose as reported by Schwimmer (*7*), and the reversibility of enzymic hydrolysis of dextrins as observed by Stark (*9*). The small molecular size of this unfermentable carbohydrate suggests that it might consist partly or entirely of isomaltose (6-[α-D-glucopyranosyl]-D-glucose), which has been isolated by Montgomery *et al.* (*5*) as one of the end products of prolonged starch hydrolysis by taka-amylase, although these authors obtained no isomaltose from maltose. Studies to elucidate the chemical nature of the product, as well as details of the enzymic reaction, are in progress.

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Activation of Arginase *in Vitro* by Mouse Carcass Extract and the Cobalt Ion

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While pursuing another phase of work on the enzyme arginase, it was observed that there might be a sex-linked difference in tissue activity in addition to general tissue variables observed by Greenstein (*1*). Further, it appeared possible that a sex-linked difference could be due to the presence in tissue of an activator other than cobalt.

In this study, the arginase activity was determined according to the method of Mohamed and Greenberg (*3*), using cobalt as metal activator, at a temperature of 40° C, for a period of 30 min. All values are the result of triplicate determinations. As seen in Table 1, the average value for 9 standard preparations of young female bovine livers, with cobalt as the activator, amounts to 377 arginase units; the average of 6 young castrated male bovine livers (cobalt-activated) amounts to 242 arginase units.

TABLE 1

Type of enzyme preparation	Type of activator	Avg arginase units* (*1*) (±2)	Percentage original units†
Young ♀ bovine liver	Co^{++}	377	100
	♂ extract	193	51
	♀ extract	168	45
	none	35	9
Young castrated ♂ bovine liver	Co^{++}	242	100
	♂ exract	176	73
	♀ extract	113	47
	none	35	14

* Per unit of standard preparation. Nine ♀ bovine livers and 6 ♂ bovine livers were used to obtain these averages.
† Ratios for both enzyme preparations with respect to each type of activator.

It appeared possible that the difference noted might be sex-linked. To test this possibility, arginase activity was determined on each of the skinned and eviscerated carcasses of 20 female and 20 male Swiss strain mice. Approximately 1 g of each mouse carcass was ground in a Waring Blendor with 2.5 ml of pH 7.0 phosphate buffer. In Table 2 there is observed approximately a fourfold difference between male and female mouse carcasses in arginase activity when cobalt is used as an activator. In the absence of cobalt there is practically no activity and no difference between male and female mouse carcasses.

In Table 1 it can be seen that a mixture of mouse carcass extract (male or female) and bovine liver arginase, in the absence of added cobalt, shows a value many times higher than either tissue preparation alone. A substance or substances in one of the tissue preparations (liver or carcass) serve as an activator for the enzyme in the other preparation. Since the standard liver preparations represent a partially purified solution

TABLE 2

Type of enzyme preparation	Type of activator	Avg* arginase units per gram of mouse carcass (±2)
♂ Mouse extract	Co^{++}	330
♀ Mouse extract	Co^{++}	90
♂ Mouse extract	none	11
♀ Mouse extract	none	12

* Average computed from determinations made on 20 ♂ and 20 ♀ Swiss-strain mice.

of the enzyme, it seems reasonable, for the present, to attribute the role of activator to the unpurified carcass preparations. Why such a carcass activator does not activate the carcass arginase cannot be stated at this time.

It seems probable that there is present in tissue an activator (or deactivator of an inhibitor) of arginase that may control the rate or degree of activation *in vivo*. Perhaps such an activator may play a role in the control

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.