supposed that extra standing rendered altered sperm completely incapable of initiating cleavage, which is for many reasons unlikely, it appears that in these experiments colchicine did *not* act by irreversible alteration of anything in the sperm prior to fertilization.

Summary. Eggs of the grass frog manifest various developmental abnormalities after fertilization with sperm previously exposed for 3 hours to 0.04% colchicine solutions. Experimental evidence is presented to show that this is not due to direct diffusion of colchicine into the egg from the sperm water nor to irreversible alteration of anything in the sperm prior to fertilization.

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The Citric Acid and Aconitase Content of the Prostate.

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The prostatic tissue has many properties of special interest. The human prostatic fluid is remarkable for its high content of citric acid and acid phosphatase, while the dog's prostatic fluid is poor in citric acid.[1-3] The growth of the tissue is greatly dependent on the sex hormones which also take part in the regulation of the oxidative phase of carbohydrate metabolism.[4] The human prostate is unable to utilize citric acid and α-ketoglutaric acid. The dog's prostate utilizes both acids, although not as much as the kidney or the heart. Synthesis of citric acid occurs in both tissues.[5]

The large amounts of citric acid found in human prostatic fluid seem to be produced by the prostate as indicated by the large citric acid content of the tissue. As can be seen in Table I, the citric acid content of human hypertrophic adenoma was about 645 mg per 100 g of fresh tissue, the values fluctuating between 1533 and 201 mg. The citric acid content in prostatic cancer was decreased by a factor of almost 10, to about 74.5 mg. The dog's prostate was found to be quite low in citric acid, about 8 mg per 100 g of tissue, *i.e.* 80 times less than the citric acid content of human prostate.

It seems that one of the causes for the large amount of citric acid in human prostate is the presence of powerful transaminations leading to the formation of oxaloacetic acid. The condensation of this dicarboxylic acid with pyruvic acid would give cisaconitic acid which in the presence of aconitase would be hydrated into citric acid. For the formation of citric acid by this process, the prostatic tissue would have to be rich in transaminase and in aconitase. Indeed there is vigorous transamination in the prostatic tissue.[5] Fig. 1 shows that the human prostate is also rich in aconitase, the dog's prostate containing only about half as much. Aconitase was extracted from the tissues by grinding with sand and 5 volumes of 0.1 *M* phosphate of pH 7.4. The suspension was centrifuged and the clear supernatant fluid was diluted so that it contained around 5 mg of tissue. To 4 cc of this solution was added 4 cc of 0.92 *M* cisaconitic

1 Schersten, B., *Skand. Arch. Physiol.*, 1936, **74**, Supp. 9.

2 Dickens, F., *Biochem. J.*, 1941, **35**, 1011.

3 Huggins, C., and Neal, W., *J. Exp. Med.*, 1942, **76**, 527.

4 Barron, E. S. G., and Huggins, C., *J. Urol.*, 1944, **51**, 630.

5 Barron, E. S. G., and Huggins, C., *J. Urol.*, 1946, **55**, 385.

TABLE I.
Citric Acid Content of Prostatic Tissue.
(Mg per 100 g of tissue).

	Human	Dog
Benign hypertrophy	218	6.3
	589	8.1
	1533	3.3
	523	9.0
	614	12.0
	201	7.0
	720	5.0
	566	10.0
	462	
	1167	
	915	
	1006	
	331	
	426	
	414	
Cancer	105	
	44	
	12	
	137	

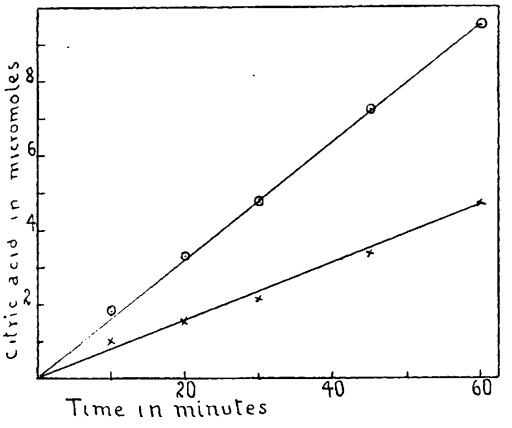

FIG. 1.
Aconitase in prostatic tissue. Enzyme extracted with phosphate buffer of pH 7.4. Cisaconitic acid concentration, 2.3 × 10^{-2} *M*. Incubation, 37.5°. Abscissa, incubation time in minutes; ordinate, citric acid formation in micromoles. The enzyme solution was that contained in 4 mg of fresh tissue.

acid neutralized in ice-cold water with solid $NaHCO_3$, 4 cc of 0.1 *M* phosphate buffer of pH 7.4 and 4 cc of water. The samples were incubated at 37.5° and the citric acid formed was determined at different intervals for 60 minutes. As the amount of cisaconitic acid was in excess, and the enzyme concentration was low, citric acid formation was linear. In 60 minutes 9.5 micromoles of citric acid were formed by the aconitase from human hypertrophic prostate; aconitase from dog's prostate produced 4.7 micromoles. According to Johnson[6] the aconitase content of tissue extracts, expressed as cmm citric acid formed per mg dry weight per hour (Q aconitase) is as follows: pigeon breast muscle, 120; liver (rat) 61.5; kidney (rat) 80; testis (rat) 7.6; lung, (rat) 14.5; brain, 10. The Q aconitase value of human hypertrophic prostate was 74 and that of dog's prostate, 43. The aconitase content of human prostate is thus almost as high as that of kidney and 10 times as high as that of testis.

The lack of citric acid oxidation, the powerful transamination, and the high aconitase content contribute to the accumulation of citric acid in the human prostatic tissue.

Summary. The citric acid content of human prostatic adenoma was about 645 mg per 100 g of tissue; that of human prostatic cancer, 74 mg; and that of dog's prostate, 8 mg. The human prostate was found rich in aconitase while that of dog's prostate contained half as much of this enzyme. The source of citric acid in prostatic tissue has been postulated.

[6] Johnson, W. A., *Biochem. J.*, 1939, **33** 1046.