Seminal Amino Acid and Carbohydrate Pattern of Bulls with Normal and Abnormal Testes Function.* (19771)

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It has been adequately demonstrated by Mann and coworkers(1-4) that elaboration of fructose by the accessory sex organs ceases after castration and that it is readily resumed following the administration of testosterone. We were able to confirm and extend their findings(5,6). In a series of transfers of sperm from normal seminal plasma to plasma of castrate animals treated with testosterone and other steroids, it was shown by us(5) that the rate of fructose utilization decreased by as much as 70% even though the seminal fructose level was within or above the normal range. This justifies the speculation that the composition of seminal plasma obtained from such animals differs from that of the intact animal either because one or more constituents are lacking or because of a shift in the quantitative relationship of some of the plasma components. Thus, it was desirable to more thoroughly characterize the nutrients in seminal plasma elaborated under various physiological conditions.

I. *Chromatography of seminal carbohydrates.* It also became apparent that as far as the carbohydrate pattern of seminal plasma is concerned, the chemical methods used have not been adequate. While Mann and his coworkers(1-3) demonstrated that the seminal sugar is not glucose but fructose, they also stated that other sugars may be present in minute quantities. Since, to our knowledge, no attempt has been made to verify these findings by more sensitive methods such as paper partition chromatography, it seemed pertinent that this procedure be employed.

Experimental. The semen or seminal plasma was extracted with 80% ethanol and the precipitated proteins were removed by centrifugation. The supernatant extract was reduced to dryness at 50°C and the dry residue diluted with 10% isopropyl alcohol to one-half the original volume. This extract concentrate was chromatographed in one dimension using the method outlined by Patton(7).

It was evident that only one sugar, namely fructose, was found in the semen of these bulls regardless of whether they were normal, vasectomized, or castrated and restituted with testosterone. It was also evident that the separation of the various sugars on the papergram is specific and critical whether this was done with mixtures of pure sugars or when such sugars were singly or in mixtures incorporated with the seminal plasma to be chromatographed. The addition of glucose to a semen sample containing fructose resulted in the appearance of 2 definite spots, glucose always being in the lower position. The concentration of sugars in spots obtained on paper following the development with either the Seliwanoff reagent or aniline hydrogen phthalate was measured with a Densichron. We were able to correlate reasonably well the fructose values obtained by chromatography with results of standard chemical analyses.

II. *Chromatography of free amino acids in bull semen.* Little is known with respect to the free amino acids of seminal plasma. Tyler and Lord Rothschild(8) offered ample evidence that amino acids play an important role in the physiology of the sex organs. For instance, they found that certain amino acids increase the life span of sperm of the sea urchin but are not used up metabolically in this relation. Lorenz and Tyler(9) showed that glycine afforded protection to sea urchin sperm against damage from ultraviolet and visible light. It has been demonstrated that the metabolism of prostatic amino acids is also influenced by testicular hormones as has been shown to be the case with seminal sugars, for Marvin and Awapara(11) found a striking variation in concentration of free amino acids in the prostate of the intact rat as compared

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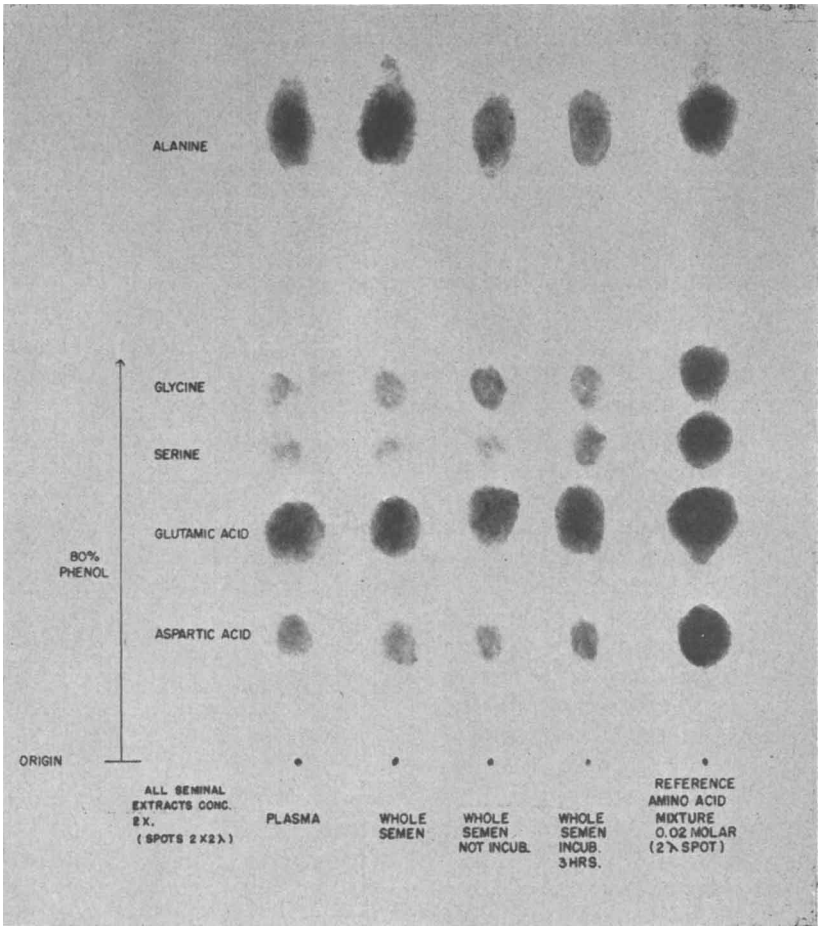

FIG. 1. Paper chromatogram of free amino acids in normal bull semen.

with the castrated animal. They further observed that androgen therapy caused a return of prostatic amino acids but the normal distribution pattern was not attained. Awapara(12) later indicated possible pathways of amino acid metabolism in the rat prostate. Thus, it seemed pertinent to examine the free amino acid pattern of semen from normal bulls and from animals which had been subjected to various experimental alterations of the reproductive system.

Experimental. A. *Free amino acid content of normal bull semen.* Seminal plasma or whole semen was extracted by a method similar to that used for the carbohydrates(13). The extract concentrate was chromatographed in 2 dimensions according to the method of Williams and Kirby(14) for qualitative estimation of free amino acids. Since separation of these amino acids was subsequently accomplished in one dimension using 80% phenol this was used thereafter.

Fig. 1 shows a typical one dimensional chromatogram of the 5 free amino acids found in normal bull semen. In the order of decreasing concentration they are glutamic acid, alanine, glycine, serine, and aspartic acid. The last column illustrates the separation of the acids from a .02 molar reference amino acid mixture. The concentration of any one of the 5 amino acids found was about the same in plasma and in whole semen. When the latter was incubated for 3 hours in order to permit metabolism of fructose by sperm, none of these amino acids was apparently utilized. This parallels the observation of Tyler *et al.*(8).

Densitometric measurements of amino acids were employed using a photoelectric scanning

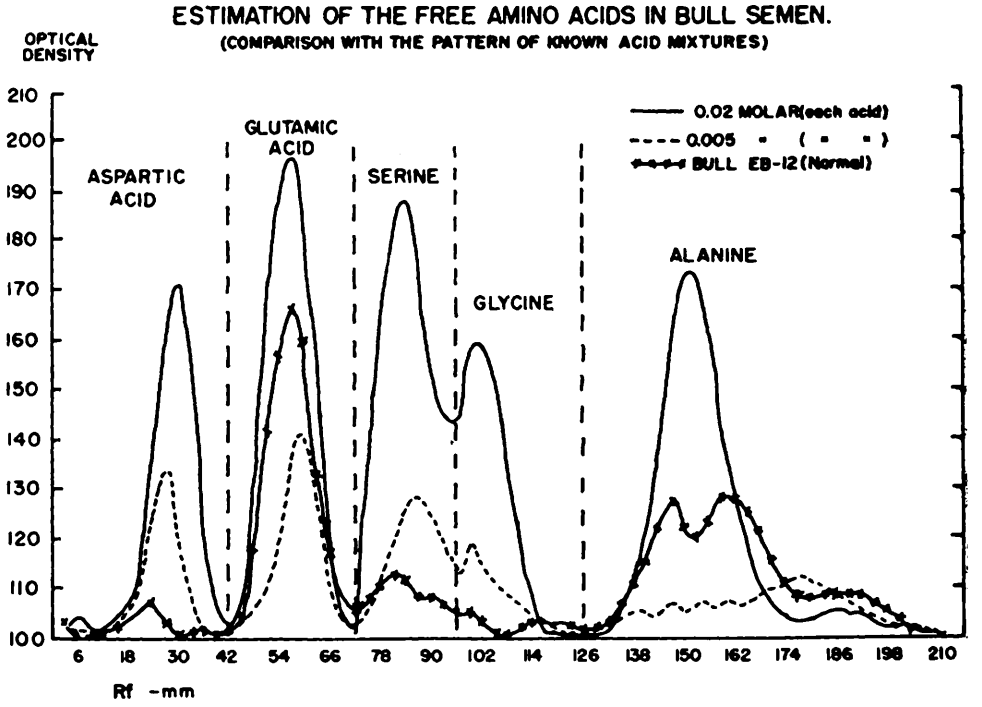

FIG. 2.

device, the Welch Densichron. The chromatographic pattern of the amino acids present in plasma specimens was measured using a paper guide, developed by Patton and Chism(15) by means of which ½ inch strips cut through the vertical axis of a finished chromatogram may be moved across the scanning probe in 3 mm increments with a 3 mm light aperture for illumination so that the strip can be studied in its entirety. The results of a typical measurement can be seen in Fig. 2. The optical densities of the various spots are plotted against the R_f in millimeters. The solid line represents the .02 M concentration of each acid while the dash line shows the concentration of the same acids at .005 M. The cross line indicates the pattern found in normal bull semen with glutamic acid showing the highest peak followed by alanine which gives a more diffused peak for both the seminal extract and the reference amino acids. The area enclosed by the curves represents the total concentration of a given amino acid in a spot. Although planimetric computation of such areas is more reliable, it is too tedious to be practical. It has been shown, however, that under properly controlled conditions the peak-density method is considerably faster and affords a reasonably accurate means of amino acid quantitation (15). After preparing standard curves for each individual amino acid the peak-height density values were translated into micromoles per 100 ml of fluid.

B. *Free amino acid content of accessory sex organ fluids in the normal bull.* In order to determine the site of production of the free amino acids found in bull semen, the seminal vesicles, ampullae of the vasa deferentia, and the epididymi of 4 normal bulls were obtained at slaughter. It was not difficult to secure up to 10 ml of seminal vesicular fluid and up to 1 ml of fluid from the ampullae. It was, however, impossible to express a sufficient amount of prostatic fluid without contamination from the tissue which surrounds this organ. The results of the estimation of amino acids are shown in Fig. 3. It is apparent that seminal vesicular fluid had an amino acid pattern similar to that found in seminal plasma with the exception that alanine and not glutamic acid was predominant. The ampullar fluid, however, showed a much higher

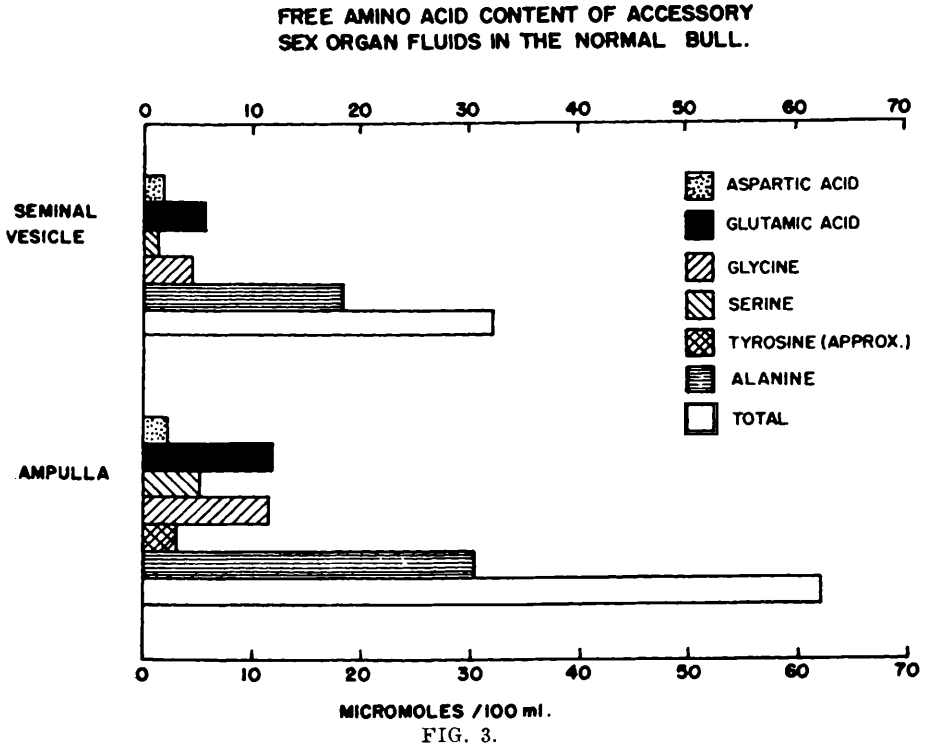

FIG. 3.

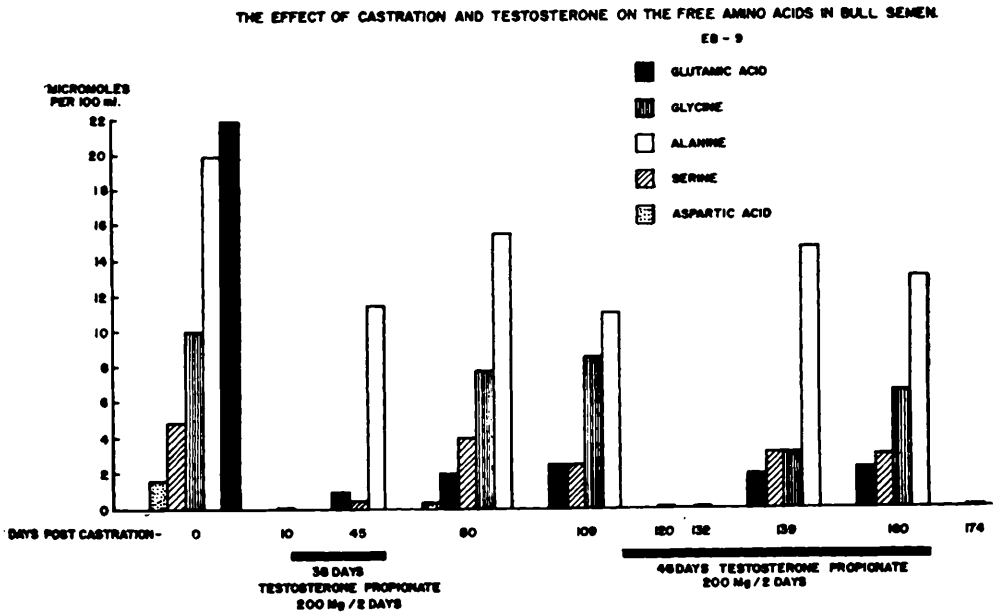

FIG. 4.

concentration in total acids per unit volume; a sixth amino acid, tyrosine, was found in 3 out of 4 specimens although in small quantity. It was not possible to obtain fluid from the epididymi in quantities sufficient for analysis.

C. *The effect of castration and testosterone*

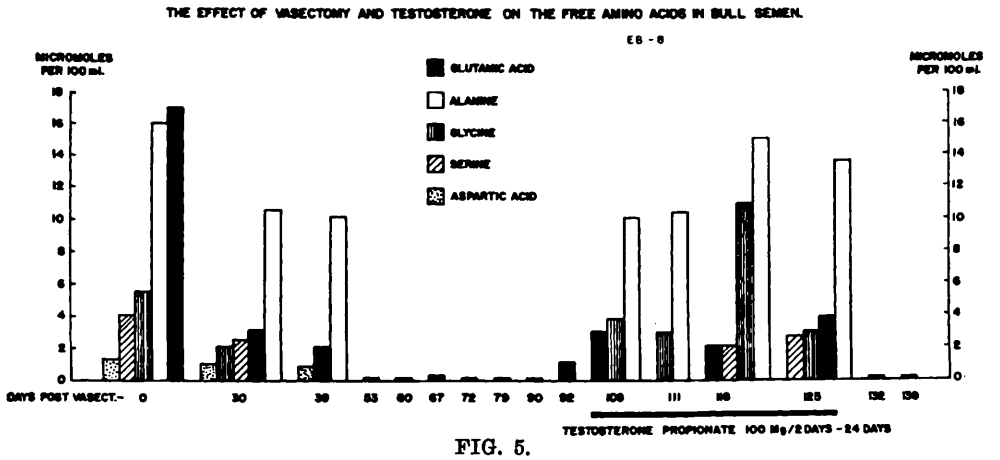

FIG. 5.

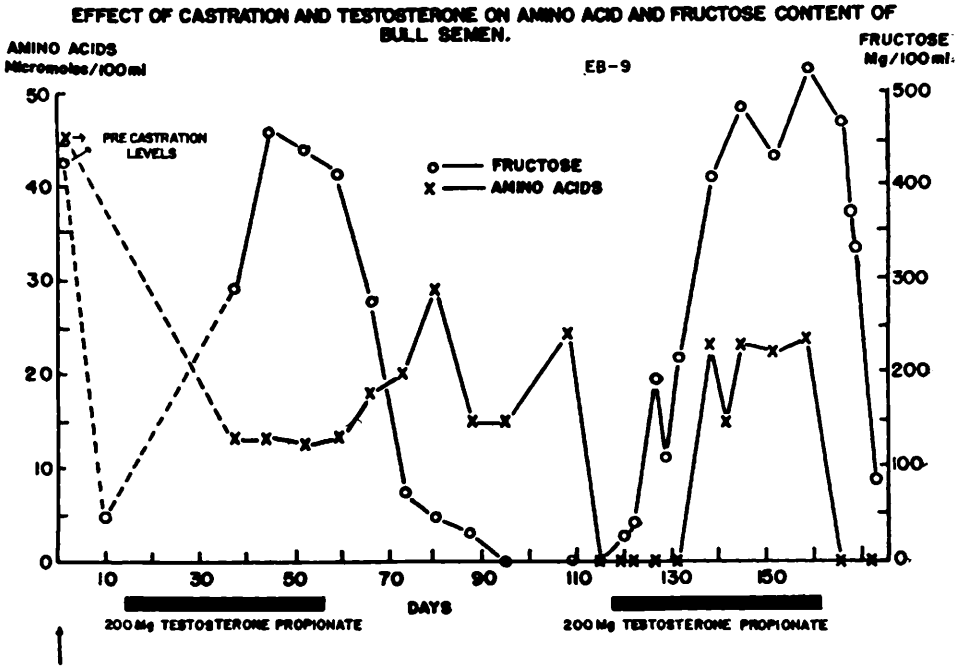

FIG. 6.

on the free amino acids in bull semen. Fig. 4 shows the effect of castration and subsequent testosterone therapy on the amino acids in normal bull semen. The 5 free amino acids disappeared within 10 days after castration. Administration of testosterone propionate (Perandren, Ciba) at 200 mg every 48 hours caused a return, within 3 weeks of alanine, glutamic acid, and serine at considerably lower levels. Glycine returned at a much slower rate while aspartic acid remained absent. Glutamic acid suffered the greatest loss whereas alanine was less affected. Although steroid therapy was discontinued after 38 days of treatment, the amino acids remained at a sub-normal level for another 51 days; thereafter, none was found. Upon the resumption of testosterone therapy for 17 days, a return of some of the amino acids was noted, but again, in no instance did this treatment result in a restitution to normal levels. The rapid loss of amino acids occurring when

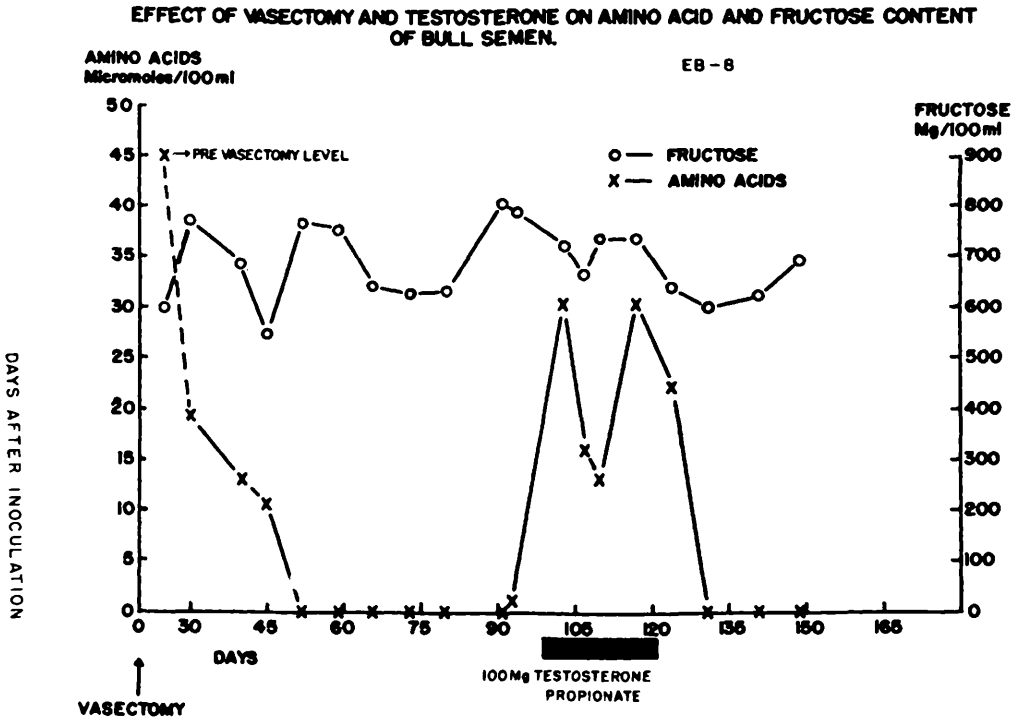

FIG. 7.

the steroid was again withdrawn is in contrast to the sluggish response of amino acids to earlier testosterone administration and withdrawal.

D. *Effect of vasectomy and testosterone on the free amino acids in bull semen.* A rather unexpected response was obtained following vasectomy. As seen in Fig. 5 the semen of this bull showed a normal amino acid concentration before vasectomy while 30 days later a marked decrease was noted; glutamic acid showed the greatest loss. Fifty-three days after vasectomy and for 2 months thereafter the seminal plasma contained no detectable amino acids. (In 2 instances trace amounts of glutamic acid were found). Testosterone propionate (Perandren, Ciba) was then administered at 100 mg every 48 hours. Seven days later the amino acids began to reappear. They returned to almost normal levels with respect to alanine and glycine but not with respect to glutamic acid. The amino acids disappeared from the plasma a week after testosterone was withdrawn. As demonstrated in Fig. 6 fructose disappeared rapidly after castration and returned with equal rapidity upon testosterone therapy. The amino acids behaved similarly but with delayed response, especially during the early post-castration period. Certainly there is a similarity in behavior of these seminal components in the castrate. Since in the vasectomized animal the testes remain *in situ* and since the testicular hormones continue to be elaborated, it could be expected that no loss in seminal sugar will occur after vasectomy. This is actually the case as shown in Fig. 7 which demonstrates that the seminal fructose remained high. Curiously, however, the free amino acids were absent by the 50th day after vasectomy. On the other hand, the administration of testosterone, while having no effect on the fructose level, resulted in a return of amino acids to near normal levels. The amino acids disappeared again when the steroid was withdrawn. Further experimentation is in progress in an attempt to explain this behavior.

Summary. 1. Methods of characterization and estimation by paper partition chromatog-

raphy of carbohydrates and free amino acids in semen and accessory sex organ fluids of the bull are described. 2. Fructose was the only sugar found in semen and in fluids expressed from the accessory sex glands. The measurement by paper chromatography agrees well with the results of chemical methods. 3. The five amino acids found in free form in semen were glutamic acid, alanine, glycine, serine, and aspartic acid. They are apparently not utilized by spermatozoa during the process of fructolysis. 4. This pattern was similar in fluids obtained from seminal vesicles and the ampullae vasa deferentia except that alanine, not glutamic acid, was predominant. A trace of tyrosine was found in ampullar fluid. 5. Seminal fructose and amino acids were completely lost after castration. While testosterone therapy caused a rapid return of seminal fructose, the normal levels of amino acids were only partially restored. 6. Vasectomy, contrary to expectations, resulted in a total depletion of seminal amino acids even though the testes remained *in situ*. That the testes continued to function normally with respect to androgen secretion was borne out by the fact that masculine behavior, sex drive and the level of seminal fructose were not affected by vasectomy. 7. Treatment with testosterone propionate, however, restored seminal amino acids to significantly higher levels. Seminal fructose remained unchanged.

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Effect of Castration and Steroid Therapy on Seminal Plasma with Respect to Fructose Utilization by Normal Bull Sperm.* (19772)

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The demonstration by Mann(1) that fructose and not glucose is the glycolyzable reducing sugar present in mammalian semen stimulated considerable interest in sperm physiology from a new and different viewpoint. Seminal fructose is elaborated by the accessory male reproductive organs under the control of the testis hormones and represents the principal nutrient substrate for the metabolic activity of spermatozoa. This was shown in various animals by Mann and coworkers(2-7). These authors were able to show that fructose

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