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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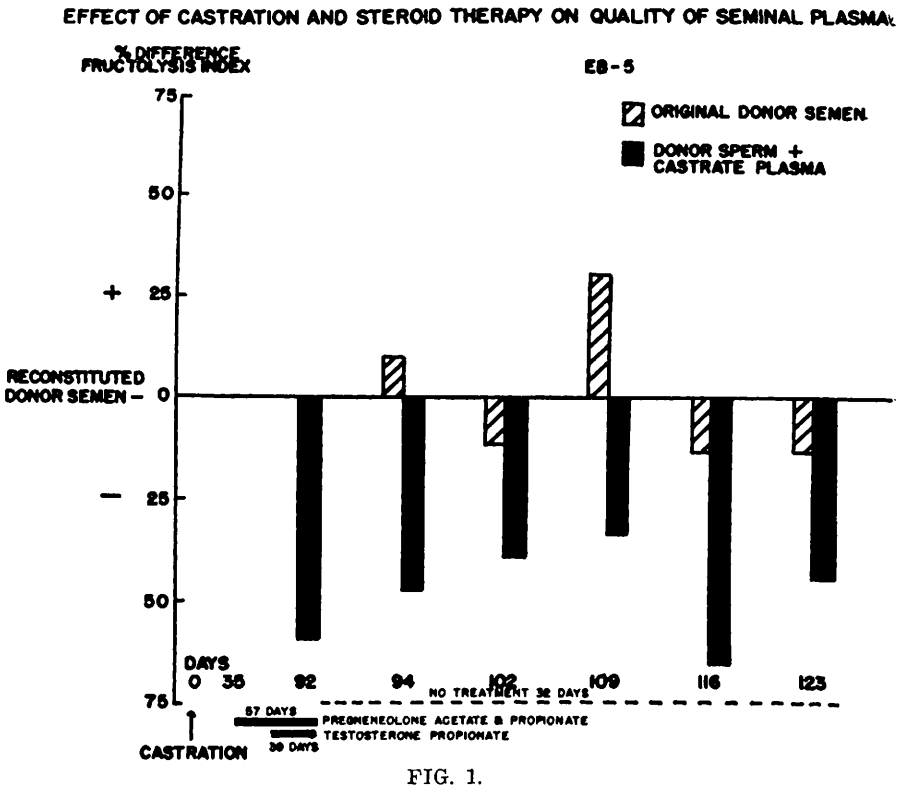

FIG. 1.

disappears from seminal plasma as well as from the accessory sex organs after castration and that implantation or injection of testosterone restores the normal fructose levels.

Gassner *et al.*(8,9) confirmed and extended these findings with respect to the utilization of fructose by spermatozoa, the effects of castration and restitution with androgenic and estrogenic steroids on seminal fructose of the bull, and the effects of gonadotrophins on the fructolysis index of sub-fertile breeder bulls.

Although the restitution of the accessory sex organs of the castrated bull is rapid and apparently complete, relative to size of the glands and secretion of fructose and citric acid, the question arises: Is the seminal plasma of the hormone stimulated castrate similar in composition to that produced by the intact animal and is it capable of supporting normal sperm metabolism? It is the purpose of this paper to report on the investigation of this problem.

Experimental. The procedure and results of a preliminary trial(9) were briefly as follows. The analytical methods were those reported by Gassner *et al.*(8).

A semen sample obtained from a normal bull was separated into 3 aliquots. One aliquot was retained as a control. Two aliquots were separated into plasma and sperm by centrifugation. The sperm residues were washed twice with phosphate buffer solution. One sample of reconstituted semen was obtained by combining one sperm residue with normal plasma in original proportions, while a second sample was obtained by combining the sperm residue with plasma of high fructose content from a bull which had been orchidectomized 3 months previously and was under treatment with pregnenolone and testosterone in an attempt to alleviate castration changes (9, p. 194). These sperm transfers were performed when the fructose content of the castrate plasma ranged from 600 to 800 mg %. The normal semen aliquot and the two reconstituted semen samples were then analyzed for fructose content, sperm concentration, and rate of fructolysis as outlined

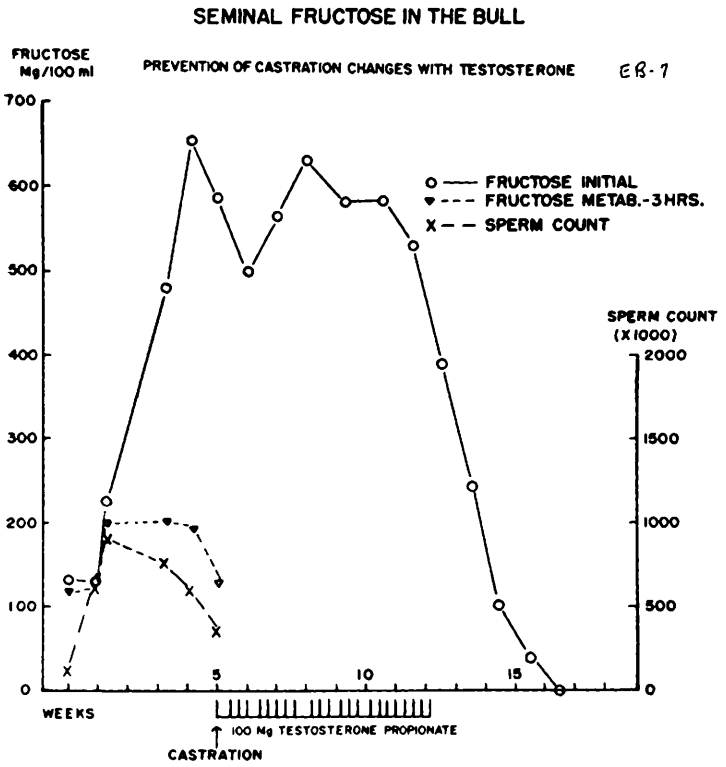

FIG. 2.

by Mann(11). In 6 consecutive weekly trials, it was found that the fructolysis index of the donor sperm-castrate plasma was 35 to 68% less than that of the donor sperm-normal plasma. (Fig. 1).

In subsequent work, the question arose as to whether semen from different individuals may be intermixed with compatibility to allow normal metabolism of fructose by spermatozoa. Semen samples from 3 normal bulls were analyzed individually and in a mixture of equal proportions for sperm concentrations, fructose content, and rate of fructolysis. The mixed semen sample was separated into plasma and sperm fractions by centrifugation. The plasma mixture and sperm mixture were then combined in original proportions. The fructolysis index† for each bull was found to be 2.83, 3.50, and 2.80, resp., with 3 hr incubations, for an average of 3.17. The index for an aliquot of the whole semen mixture was 3.17. The index for the reconstituted semen mixture (sperm mixture plus plasma mixture) was 3.50. Thus, there was no change in activity due to mixing of semen from different donors. It was also demonstrated that the procedure employed in sperm transfer has no effect on the fructolysis index.

It appears that, since normal sperm were not able to utilize fully the fructose in seminal plasma of castrates, the chemical composition of plasma from castrates restituted with certain steroids differs from the seminal plasma produced by the intact bull in some manner. These observations further suggest that the normal testis may elaborate hormonal substances other than testosterone and estrogen or that the epididymal fluid may contribute nutrients which are not furnished by the accessory sex organ fluids, all of which are required by spermatozoa for the maintenance of normal fructose metabolism. In an attempt to shed further light on this problem the following experiments were conducted.

† (Fructolysis index: mg. fructose metabolized in 3 hr/10^9 sperm cells).

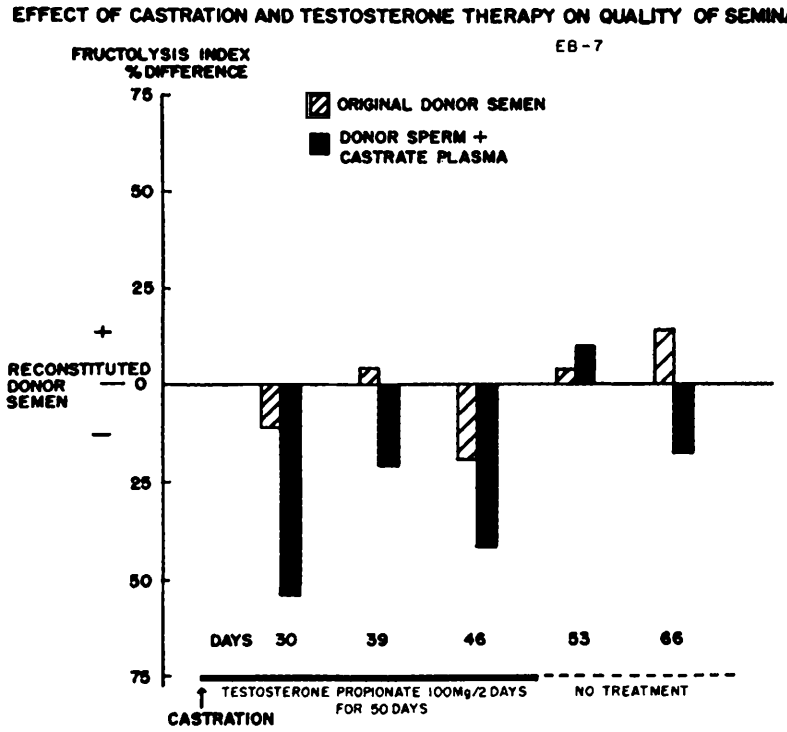

FIG. 3.

1. *Effect of castration and maintenance with testosterone on seminal plasma quality.* A normal bull was castrated and received, on the same day and every 2 days thereafter for 50 days, 100 mg of testosterone propionate (Perandren, Ciba). The seminal fructose level remained high until a week after testosterone was withdrawn whereupon it rapidly declined (Fig. 2). Normal bull sperm transferred at weekly intervals to plasma obtained from this castrate failed to utilize as much fructose as the sperm combined with normal plasma (Fig. 3).

2. *Effect of castration and restitution with testerone on seminal plasma quality.* A second normal bull was castrated. Supporting steroid therapy was delayed in order to allow the accessory sex glands to regress in size and function. After seminal fructose had dropped to zero, 200 mg of testosterone propionate (Perandren, (Ciba) was injected every 2 days. Seminal fructose returned rather rapidly and disappeared soon after the steroid was withdrawn. Following an 8-week rest period testosterone therapy was resumed with the result that the seminal fructose again rose to the precastration level. Upon cessation of the therapy, fructose again disappeared (Fig. 4). Seminal plasma of this bull was tested repeatedly to determine its ability to support the utilization of fructose by normal sperm (Fig. 5).

Two weeks after castration and before treatment with testosterone was begun, seminal fructose had declined to 27 mg %. When 400 mg % fructose were added, only 20% of the fructose present was metabolized by the donor sperm. Testosterone seemed to have an immediate effect on the seminal plasma since the rate of fructolysis was greater than that shown by the reconstituted donor semen. Upon withdrawal of the steroid, only 30% of the fructose present was utilized. The effect of resumption of steroid therapy is shown in that fructose was again more efficiently metabolized. Thereafter the seminal plasma quality seemed inadequate to fully support fructolysis by normal sperm. It should be pointed out that 5 months had elapsed since castration and that the response seen may indicate a loss in target organ re-

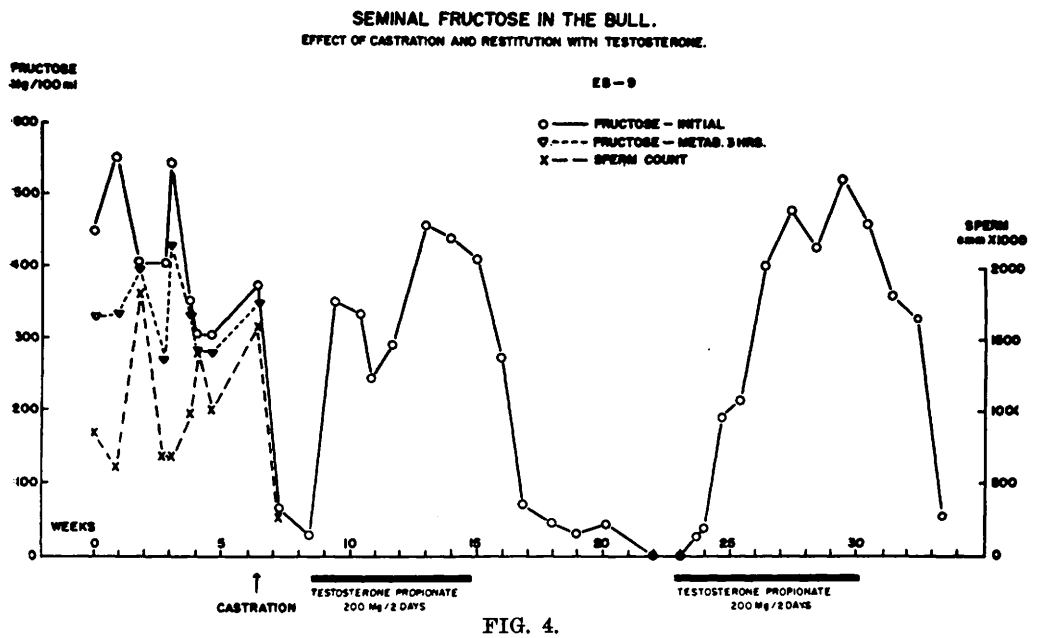

FIG. 4.

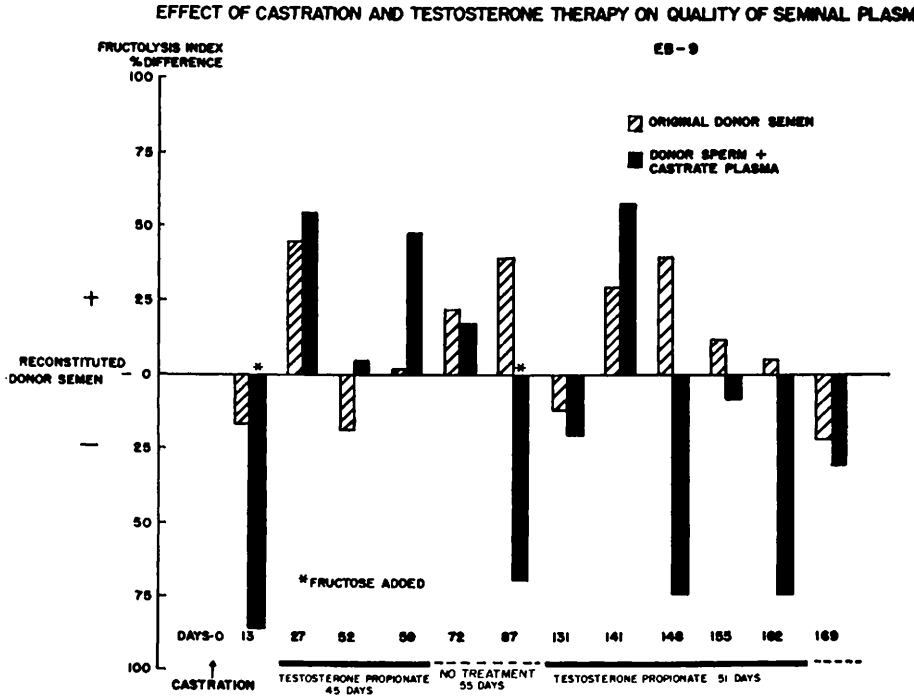

FIG. 5.

sponse as observed earlier(9).

3. *Effect of vasectomy and testosterone on the quality of seminal plasma.* Since in the vasectomized animal the testes remain *in situ*, the accessory sex glands remain under the trophic influence of the testis hormone and should produce a normal plasma. Therefore, it should behave in a similar fashion as normal seminal plasma deprived of sperm by centrifugation. To test this assumption vasore-

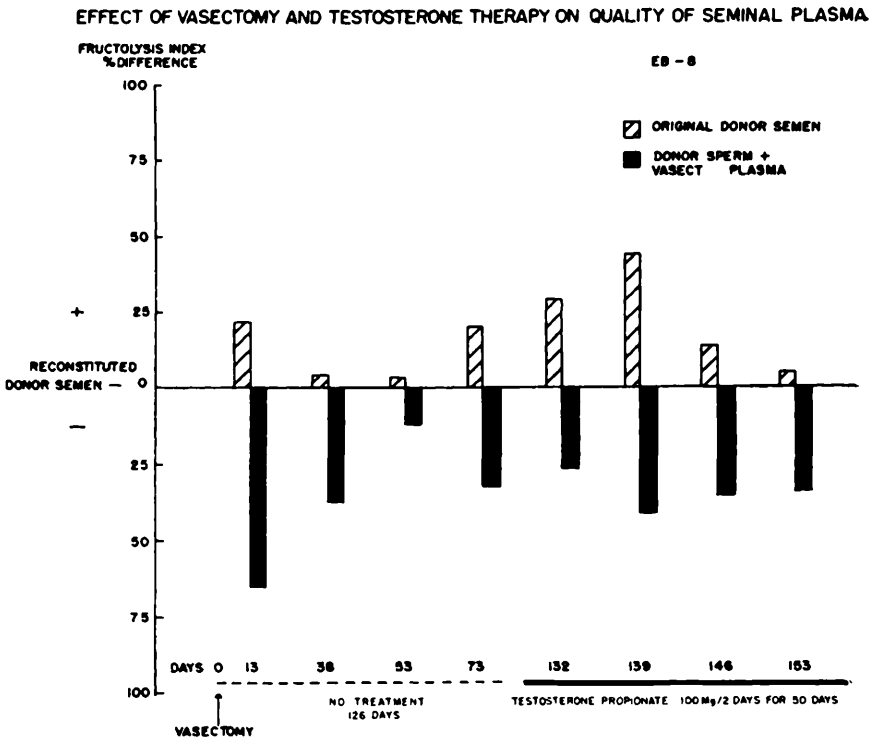

FIG. 6.

section was performed on a normal bull. The seminal plasma was analyzed for fructose concentration at weekly intervals. The fructose content was in no way affected by vasectomy nor was it changed when testosterone propionate was given for a period of over four weeks. Normal bull sperm were transferred repeatedly to plasma obtained from this animal. While during the first 2 months after vasectomy the degree of fructose utilization by normal sperm gradually improved, it remained below normal thereafter regardless of the fact that testosterone propionate was given (Fig. 6).

Discussion. At the moment it is impossible to draw any definite conclusions. It appears that testosterone cannot fully compensate for castration changes brought about in the accessory sex organs, particularly as far as the chemical composition of their secretions is concerned. The response shown by the vasectomized animal is puzzling. If the evaluation of the role of the governorship exerted by the testes over the accessory glands is correct, then there should be no difference in the metabolic behavior by normal sperm when reconstituted with either normal plasma or with plasma delivered by the vasectomized bull. A similar response to vasectomy was shown with respect to other seminal components, namely, the free amino acids(10). We have been able to show that the free amino acids disappear completely after vasectomy but return, at least in part, when testosterone propionate is injected. It has been further shown by us that in the normal bull the amino acid pattern of fluids obtained from the ampullae vasa deferentia is quite similar to that of the seminal vesicles. This justifies the tentative conclusion that vasectomy affects the functional status of the accessory sex structures.

Another series of bulls, vasectomized or castrated and receiving androgens and estrogens and mixtures thereof, are at the present time on trial. Information received so far from these experiments has confirmed the observation reported here.

Summary. 1. The method employed for

the separation of normal bull semen into plasma and sperm with subsequent reconstitution appears to have no detrimental effect upon the ability of the sperm to metabolize fructose. 2. Similar results were obtained when semen from three normal bulls was intermixed. 3. The immediate administration of testosterone propionate to a bull at the time of castration prevents the loss of seminal fructose resulting from castration. Seminal plasma from such an animal was not fully capable of maintaining the metabolism of fructose by normal sperm. 4. Seminal plasma of negligible fructose content from a bull in which castration changes were allowed to develop before testosterone therapy was begun failed to support a normal rate of fructolysis since only 20 per cent of added fructose was utilized by normal sperm. Testosterone therapy caused a marked increase in both seminal fructose and the utilization thereof. Subsequent withdrawal and resumption of steroid therapy produced similar results. Thereafter, in spite of continued treatment with testosterone, the seminal plasma of this castrate only partially supported fructose metabolism. 5. Vasoresection in a bull had no adverse effect on either sex drive or the production of seminal fructose. However, when normal sperm was transferred to seminal plasma from this bull, the utilization of fructose was, contrary to expectations, markedly reduced. This would indicate that vasectomy adversely affects the functional status of the accessory sex organs.

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Development of Electrical Activity in the Cerebral Cortex of the Albino Rat.* (19773)

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Rhythmical electrical activity appears rather suddenly in the cerebral cortex of the guinea pig during the seventh week of gestation(1,2). Various morphological and biochemical changes also occur at this particular stage of development in this animal(3,4). The present study is concerned with an attempt to determine the critical period, for the albino rat, at which the cortical neurons become functionally active, using the electrocorticogram as an indicator of this development.

Methods. About 50 albino rats of various ages from the newly born to the adult were studied. Ether, diallylbarbituric acid (25-50 mg/kg, I.P.), or d-tubocurarine chloride (0.2-1 mg/kg, I.P.) were used to immobilize the animals in order to permit direct recording of electrical potentials from the cerebral cor-

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