Effect of Hyperglycemia on Fructose Biosynthesis in Mouse Seminal Vesicles.* (31895)

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Mann(1,2) first isolated and identified fructose as the principal sugar present in bull semen and demonstrated that in many species the site of production of this sugar is in the seminal vesicles. Mann and Parsons(3) showed that the presence of fructose in the semen was dependent upon testosterone. Further, Lutwak-Mann, Mann and Price(4) transplanted small pieces of sex accessory glands to host animals and noted that the transplants accumulated fructose under the influence of the male sex hormone.

Mann and Parsons(5) presented evidence indicating that blood glucose serves as the precursor for the biosynthesis of fructose by seminal vesicle tissue. They also demonstrated that rabbits made diabetic with alloxan had greater amounts of fructose in their semen than did their non-diabetic littermates. Treatment of the diabetic animals with insulin resulted in a marked decrease in the fructose content of the semen.

In vitro experiments by Mann and Lutwak-Mann(6-8) showed that fructose was formed when minced accessory gland tissue was incubated with glucose and that the minces contained all the enzymes necessary for the phosphorylative conversion of glucose to fructose. More recently Hers(9,10) and Samuels, Harding and Mann(11) have described the presence of enzyme systems in seminal vesicle tissue which convert blood glucose to sorbitol and sorbitol to fructose. An aldose reductase is responsible for the reduction of glucose to sorbitol and a ketose reductase oxidizes the sorbitol to fructose.

Thus both of the pathways so far elucidated for the biosynthesis of fructose by seminal vesicle tissue appear to be dependent upon blood glucose levels as well as the presence of testosterone. The present study was designed to investigate in the living animal the effect of increased glucose on the production of fructose by mouse seminal vesicle under the stimulus of known amounts of testosterone.

Materials and methods. The animals used in these experiments were adult C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine). All were castrated and their seminal vesicles allowed to regress for from 30 to 35 days. During this period the basal glucose level of each animal was determined on blood obtained from the orbital sinus after fasting each mouse for 15-17 hours. Glucose was determined by the use of glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N. J.). At the end of the period allowed for regression of the sex accessory organs one-half of the mice were randomly selected and made hyperglycemic by injection with 100 mg/kg of alloxan monohydrate into the external jugular vein. Forty-eight hours after administration of the alloxan the basal blood glucose level was again determined. Only animals whose blood glucose level had increased at least 100% above the previous basal level were considered to be diabetic and included in the experiment. Once the hyperglycemia was established, all mice, diabetic and non-diabetic, were given daily subcutaneous injections of 100 μg of testosterone propionate. The animals were sacrificed on 0, 2, 4, 8 and 12 days after the start of hormone injections. Blood glucose levels were determined just prior to sacrifice as a check on the hyperglycemic condition. The animals were killed by cervical dislocation and the seminal vesicles removed, separately from the coagulating glands, cleaned of surrounding tissues and lyophilized after freezing in precooled aluminum containers packed in powdered dry ice. Dry weights were obtained from the frozen-dried material.

In addition to the above experiments a group of animals which had been castrated

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TABLE I. Effect of Hyperglycemia on Freeze-dried Weights of Seminal Vesicles of Castrate Mice Receiving Testosterone Propionate Therapy for Varying Periods.

Intact control (mg)	Days of treatment with testosterone propionate	Castrate + testosterone propionate non-diabetic	Castrate + testosterone propionate diabetic
—	0	11.2 ± 3.6	7.5
—	2	11.9 ± 3.1	6.0 ± 1.3
—	4	12.6 ± 3.1	9.8 ± .6
—	8	26.2 ± 2.5	31.2 ± 2.1
66.4 ± 2.20	12	65.1 ± 3.4	57.4 ± 2.0

Adult C57BL/6J males were castrated. Thirty to thirty-five days later one-half of them were made diabetic by intravenous injections of alloxan monohydrate. Animals were considered to be hyperglycemic when blood glucose increased at least 100% above basal level. All animals were given daily injections of 100 μg of testosterone propionate and individuals sacrificed on days 0, 2, 4, 5 and 12 of hormone treatment. Seminal vesicles were removed, freeze-dried, weighed and analyzed for fructose. All results are expressed in mg representing mean weight of seminal vesicles ± one standard error. All groups contained 6 or more animals except the one for day 0-diabetic which was composed of 2 individuals.

for 4 months were examined to see if an induced hyperglycemia in the absence of male sex hormone would increase fructose production. Half of the animals were made diabetic with alloxan, and their blood glucose levels were determined using the techniques previously described. Twelve days after the diabetic condition was established the animals were sacrificed and the fructose content of the seminal vesicles determined and compared with the values obtained from the seminal vesicles of the remaining control animals which had been castrated but not made diabetic.

The seminal vesicles were homogenized in distilled water and deproteinized with Ba $(OH)_2$ and $ZnSO_4$. The fructose content of the supernatant was determined by the method of Roe(12) as modified by Linder and Mann(13).

Results. The relationships of the dry weights of the seminal vesicles from the castrate, diabetic and testosterone propionate-treated animals are compared with those from the castrate, non-diabetic, testosterone propionate-treated individuals in Table I. When the weights of the seminal vesicles from the two experimental groups are compared with those from a group of normal intact mice of the same age group the expected effect of 30-35 days of castration is demonstrated by the marked reduction in dry mass. No effect of testosterone propionate therapy, as reflected by weight changes, was observed until the marked increase noted by the eighth day. By the twelfth day the weight of the seminal vesicles from all experimental animals had returned to a mass approximating that of the intact non-treated control group. In no instances did the response of the castrate, non-diabetic, testosterone propionate-treated animals differ from that of the castrate, diabetic, testosterone propionate-treated mice.

Table II shows the fructose concentrations found in these same seminal vesicles. Following the onset of testosterone propionate therapy an immediate and continued increase above the basal castrate, non-treated levels was observed for both experimental groups. By day 4 the fructose concentrations in the seminal vesicles of the castrate, diabetic, testosterone propionate-treated individuals were

TABLE II. Effect of Hyperglycemia on Fructose Content of Seminal Vesicles of Mice Receiving Testosterone Propionate Therapy for Varying Periods.

Intact control (mg)	Days of treatment with testosterone propionate	Castrate + testosterone propionate non-diabetic	Castrate + testosterone propionate diabetic
—	0	1.25	2.07
—	2	2.17 ± .58	2.82 ± .64
—	4	1.97 ± .12	7.20 ± 1.75
—	8	9.20 ± .70	21.22 ± 1.45
15.10 ± .55	12	15.06 ± .59	27.61 ± .99

Adult C57BL/6J males were castrated. Thirty to thirty-five days later one-half of them were made diabetic by intravenous injections of alloxan monohydrate. Animals were considered to be hyperglycemic when blood glucose increased at least 100% above basal level. All animals were given daily injections of 100 μg of testosterone propionate and individuals sacrificed on days 0, 2, 4, 8 and 12 of hormone treatment. Seminal vesicles were removed, freeze-dried, weighed, homogenized in distilled water, deproteinized and fructose content of the supernatant determined. All results are expressed in μg of fructose/mg of freeze-dried seminal vesicle ± one standard error. All groups contained 6 or more animals except the 2 values for day 0 which are composed of data from 2 individuals in each group.

TABLE III. Effect of Hyperglycemia on Freeze-dried Weights and Fructose Content of Seminal Vesicles of Castrate Mice Receiving *No* Testosterone Therapy.

	Weight seminal vesicles	Concentration of fructose
Castrate-non diabetic	2.46 ± .63	5.09 ± 1.38
Castrate diabetic	1.89 ± .15	3.28 ± .15

Adult C57BL/6J males were castrated. Approximately 4 months later individuals were made diabetic by intravenous injections of alloxan monohydrate. Animals were considered to be hyperglycemic when blood glucose increased at least 100% above base level. On day 12 after establishment of diabetes the animals were killed, seminal vesicles were removed, freeze-dried, weighed and analyzed for fructose. All results are expressed in mg ± one standard error for weight and μg of fructose/mg of freeze-dried seminal vesicles ± one standard error for fructose concentration. Each group contained data from at least 4 individuals.

significantly greater than those observed in the castrate, non-diabetic, testosterone propionate-treated mice. By day 12 the diabetic animals exhibited fructose concentrations nearly twice those observed in the non-diabetic mice. It is interesting to note that the concentration of fructose in this latter group almost exactly reproduces the level observed in the intact, non-treated population.

The data presented in Table III indicate that in the absence of testosterone therapy the castrate, diabetic animals did not produce significantly greater amounts of fructose when compared with animals castrated for similar periods of time, given no hormone therapy but having normal blood sugar levels. The difference in weights of the seminal vesicles observed in this experiment and the preceding experiment is a function of the much longer period of time allowed for regression of these organs in this group of mice.

Discussion. In the 2 groups of mice both of which were castrated and received similar amounts of testosterone propionate those animals which were also made hyperglycemic exhibited a greatly increased synthesis of fructose when compared with animals having normal blood sugar levels. We must conclude that increased substrate in the presence of hormone results in increased biosynthesis of fructose. In contrast, those mice which were castrated and made hyperglycemic but received no testosterone propionate showed no significant increase in fructose production by the seminal vesicles when compared to castrate controls with normal blood glucose. Such data indicate that even in the presence of excessive levels of glucose there is no demonstrable conversion of this substrate to fructose unless testosterone is present.

In the *in vivo* situation the male sex hormone appears to be essential for the enzymatic conversion of glucose to fructose by the mouse seminal vesicle. However, when Samuels, Harding and Mann(11) examined these relationships *in vitro* they were unable to demonstrate any direct action of testosterone on the specific enzyme systems known to be involved in the conversion. Rather they suggest a broader role for the hormone, one in which it stimulates enzyme synthesis generally, so that aldose and ketose reductase activity in the seminal vesicle epithelium becomes only one of several or more reactions governed indirectly by the male sex hormone.

Summary. Male C57BL/6J mice were castrated and their sex accessory organs were allowed to regress for 30 to 35 days. At this time one-half of the castrate animals were made diabetic with alloxan. Both the diabetic and non-diabetic mice were given daily injections of 100 μg of testosterone propionate and sacrificed after 0, 2, 4, 8 and 12 days of hormone therapy. The frozen-dried weights of the seminal vesicles of the castrate, non-diabetic, testosterone propionate-treated animals did not differ significantly from that of the castrate, diabetic, testosterone propionate-treated mice. By day 12 of hormone therapy the weight of the seminal vesicles from the experimental animals had returned to a mass approximating that of the intact control group. The concentration of fructose in the seminal vesicles was significantly higher in the diabetic than in the non-diabetic mice. The concentration of fructose in the non-diabetic groups was almost identical with that observed in the intact control group. A second group of mice were castrated for 4 months, then one-half of them were made diabetic with alloxan. No hormone therapy was administered. On day 12 after the establishment of diabetes they were killed along with the castrated but non-

diabetic control group. In the absence of testosterone propionate therapy diabetic animals did not produce significantly greater amounts of fructose than did castrated control animals with normal blood sugar levels. It appears that the male sex hormone is in some manner necessary for *in vivo* formation of seminal fructose from blood glucose, even when the latter substrate is present in excessive amounts. These data do not elucidate the mechanism whereby testosterone controls this conversion.

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Lung Lipase Levels in Normal Rats and Rats With Experimentally Produced Fat Embolism.* (31896)

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Fat embolism is a condition in which fat appears in the circulating blood, not in the fine emulsion of a metabolic lipemia, but in droplets large enough to occlude arterioles and capillaries. Recent experiments on rats in our laboratory indicate that the lung is the only organ or tissue which captures fat droplets in any significant quantity when it is injected intravenously as triolein(1). Embolic fat disappears from the lung of the rat over a period of a few days after injection(1). While some is removed by phagocytosis, it appears that the major portion is mobilized by enzymatic breakdown of the neutral fat. That the lung secretes lipase as one of its many nonrespiratory functions has been known for some time(2,3).

This experiment was designed to measure changes of lung lipase activity in rats following the injection of a measured amount of triolein. The heart, which is also known to have lipase activity(3), was used as a control organ.

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Methods. Twenty-four pairs of Holtzman male rats from 6 to 11 weeks old were used. Each pair consisted of a rat injected with 0.25 ml triolein and a rat of the same age and approximate weight injected with 0.25 ml of 0.9% saline solution. Both rats were carried together through the experimental procedure of injection, sacrifice, preparation of acetone powders, and lipase determinations.

Forty-eight hours after injection each rat was given 0.15 ml of sodium pentobarbital (65 mg/ml), the abdomen was opened and the animal was sacrificed by withdrawal of blood from the aorta. The heart and lungs were removed and placed in cold 0.9% saline.

Acetone powders of each organ were prepared, weighed and stored in a desiccator at —18°C until lipase determinations were performed.

Extracts of acetone powders were obtained for lipase assay, by mincing one-half of the acetone powder from each organ with 8 ml distilled water, shaking, incubating for 15 minutes at 37-38°C, shaking again and centrifuging. The aqueous layer was removed