STUDIES IN FRUCTOLYSIS IN HUMAN SEMEN. I EFFECTS OF SOME HORMONES AND INORGANIC SUBSTANCES

By

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It is known (Mann, 1945) that fructose is the glycolysable sugar that occurs in the semen of man and that spermatozoa are capable of utilising fructose. It has been further shown by Mann (1948, a) working with bull semen, by Davis and Macune (1950), Birnberg, Sherber and Kurzok (1952) and Vaishwanar (1958) with human semen, that fructolysis can give a good indication of the metabolic activity of spermatozoa. Studies to find out the effect of various substances on the fructolysis of human semen have been hampered for the lack of an accurate and convenient method to measure fructose and fructolysis in human semen. Recently, Sheth and Rao (1959, a) have reported an improved method for the estimation of fructose and fructolysis in human semen. This method has been used in the work reported here, to study the effect of temperature, some hormones and inorganic salts on the fructolysis in human semen.

MATERIALS AND METHODS

Semen samples were obtained from healthy donors. Spermatozoal count and motility were determined soon after liquefaction of the semen, and the same was immediately used for the experimental work.

Fructose in the control and experimental semen sample was seperated from other reducing substances by circular paper chromatography and then estimated as described in our previous communication (Sheth and Rao, 1959, a). In every chromatogram, the control semen sample and the experi mental were spotted in duplicates. A standard fructose solution was always spotted on the chromatograms and the chromatogram was developed as described before.

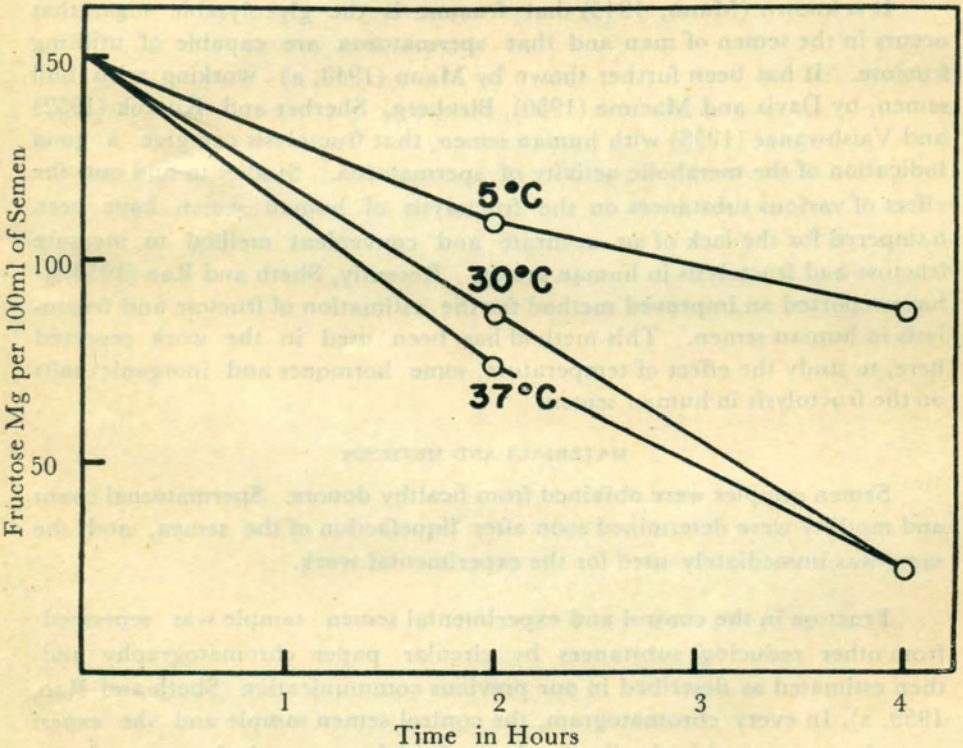
EXPERIMENTAL.

Effect of temperature on fructolysis. Temperature has long been known to exert a powerful influence on the motility and fertilising capacity of spermatozoa (Chang and Walton, 1940). Sheth and Rao (1959, b) found that the succinic dehydrogenase activity of human semen increased with sudden

lowering of temperature. It was of interest to study the effect of temperature on fructolysis in human semen.

One ml. aliquotes of a semen sample with a sperm count of 92 Million/ml. and a motility of 60 per cent was maintained at 5°, 30° and 30°C. The amount of fructose present initially and at the end of 2 hrs. and 4 hrs. was determined chromatographically. Graph I gives the results of the experiment. It is evident that fructolysis slows down markedly at lower temperature.

EFFECT OF TEMPERATURE ON FRUCTOLYSIS IN HUMAN SEMEN



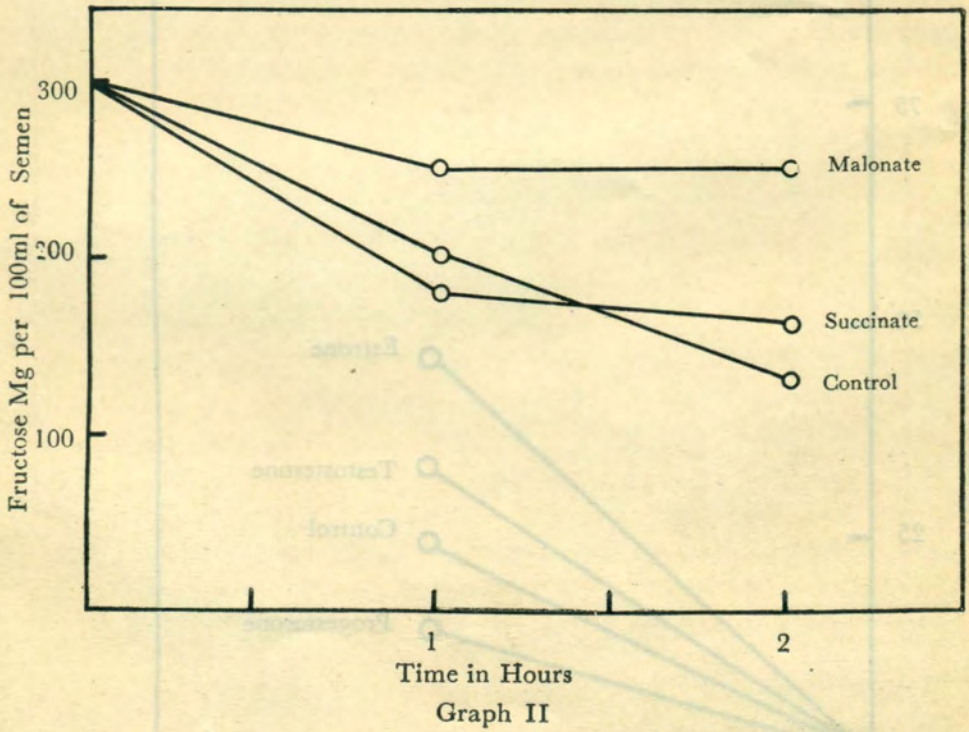
Graph I

Effect of succinate and malonate. The presence of succinic dehydrogenase in human semen was reported by MacLeod (1943). Rao and Sheth (1959) have further shown that succinic dehydrogenase activity of human semen gave a good indication of the initial percentage motility of a semen sample. MacLeod (1946) reported that addition of sodium succinate to semen decreased lactic acid production, which is indicative of a lower rate of gly-

colysis. It is well known that malonate is a selective poison for aerobic respiration. Phillips and Lardy (1941) showed that the respiration of spermatozoa is inhibited by malonate. It was therefore of interest to study the effect of succinate and malonate on fructolysis in human semen.

Two tubes A and B containing 1 ml. of semen each, succinate and malonate were added so that their final concentration was 0.05 M and 0.1 M respectively. Tube C containing only 1 ml. of semen served as control. The tubes were incubated at 37°C. At the end of 0 hr., 1 hr., and 2 hrs., 0 01 ml. quantities were pipetted out for the estimation of fructose. The results seen in Graph II show that during the first hour succinate increased the rate of fructolysis which then slowed down and during the second hour the rate was lower than in the control. It also shows that the inhibition of fructolysis is not instantaneous but is complete within two hours of incubation.

EFFECT OF SUCCINATE & MALONATE ON FRUCTOLYSIS IN HUMAN SEMEN



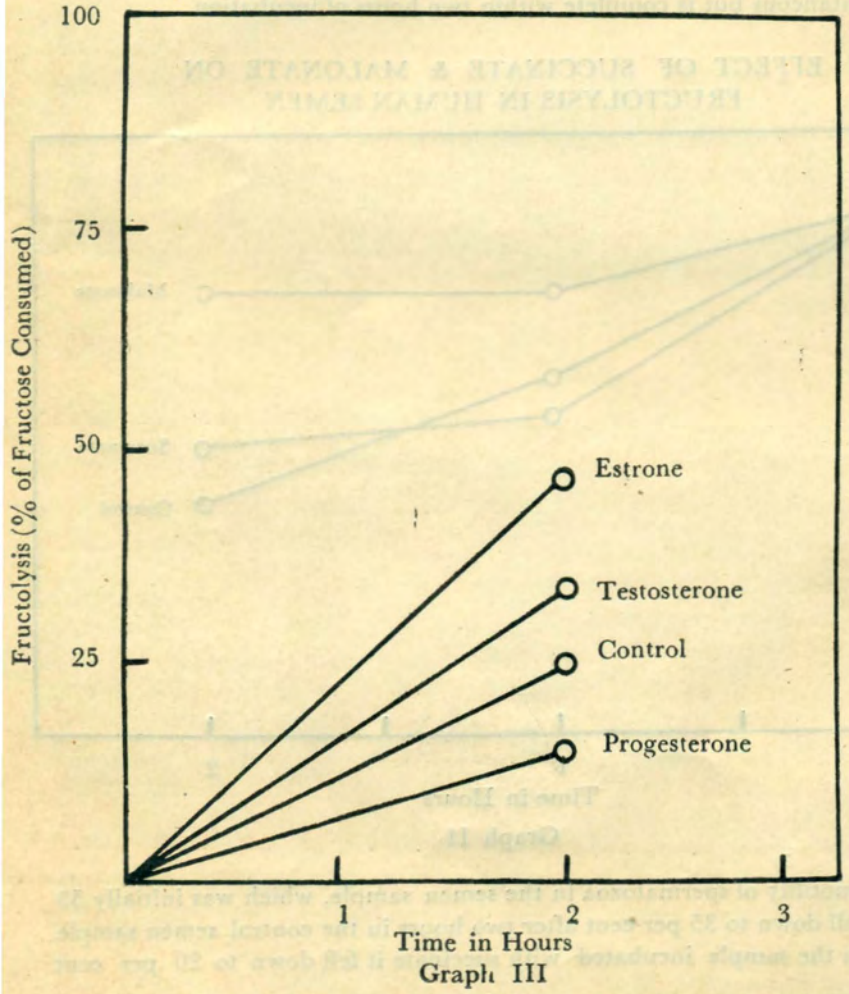
Graph II

The motility of spermatozoa in the semen sample, which was initially 50 per cent fell down to 35 per cent after two hours in the control semen sample whereas in the sample incubated with succinate it fell down to 20 per cent

In the sample treated with malonate all the spermatozoa were immotile at the end of two hours.

Effect of hormones. A 0.5 per cent solution of hormone under study was prepared in 95 per cent alcohol. 0.1 ml. of this solution was mixed with 1.9 ml. of phosphate buffer of pH 7.4., 0.2 ml. of this buffered hormone mixture was added to 1 ml. of semen. The final concentration of the hormone was 50 microgram per ml. of semen. The semen sample was incubated at 37°C. The amount of fructose utilised was determined by finding out the fructose present initially and at the end of 2 hrs. Values obtained are shown in Graph III. The graph shows that while the rate of fructolysis was acti-

EFFECT OF TESTOSTERONE, ESTRONE & PROGESTERONE ON FRUCTOLYSIS IN HUMAN SEMEN



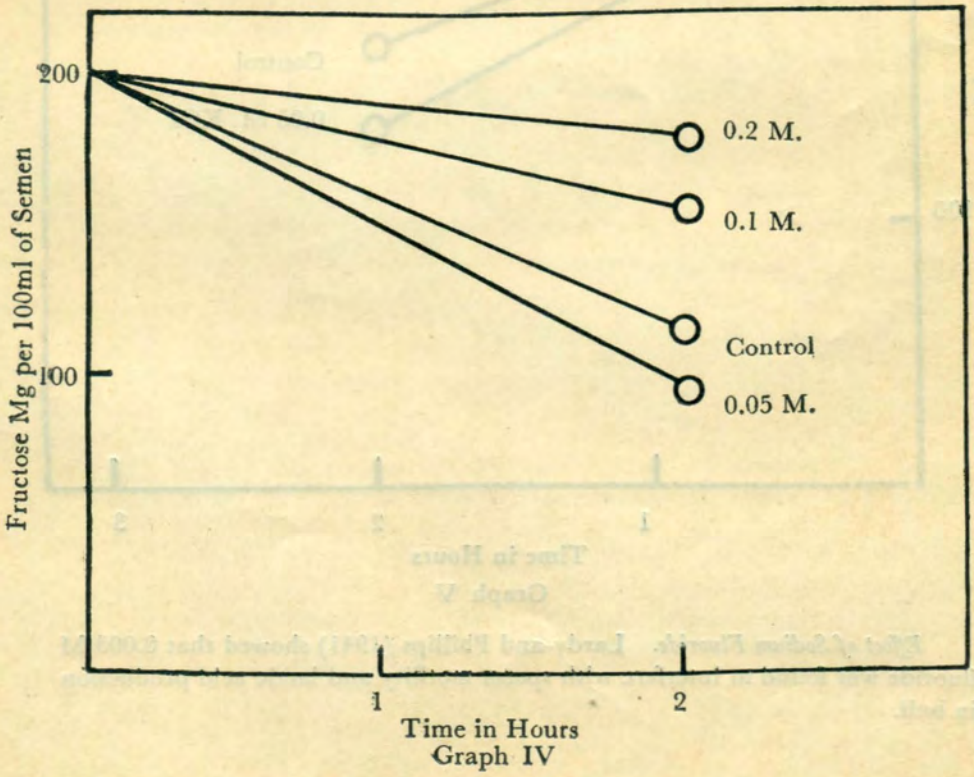
Graph III

vated by testosterone and estrone it was depressed by progesterone. Microscopic examination of semen revealed that in semen samples treated with testosterone and estrone, spermatozoa were actively motile, whereas in the semen samples treated with progesterone the spermatozoa were sluggishly motile.

Influence of Chlorides of sodium and potassium. As early as in 1853, Neuport demonstrated that sodium choride is capable of stimulating or inhibiting motility according to the concentration and specific experimental conditions. Mann (1954) in his studies on the boar vesicular secretion stressed that citric acid in combination with potassium ions and sodium ions, plays an important part in the maintenance of the osmotic equilibrium in semen.

An experiment was designed to study the effect of varying concentration of sodium chloride on fructolysis. 0.5 ml. of semen was taken in each of the tubes marked A, B, C and D. 1.7, 3.5 and 7 mg. of sodium chloride were added to tubes A, B and C respectively. The tube D served as a control. The fructose was determined at the end of two hours of incubation at 37°C Graph IV shows that with increased concentration of sodium chloride there

EFFECT OF VARYING CONCENTRATION OF NaCl ON FRUCTOLYSIS IN HUMAN SEMEN

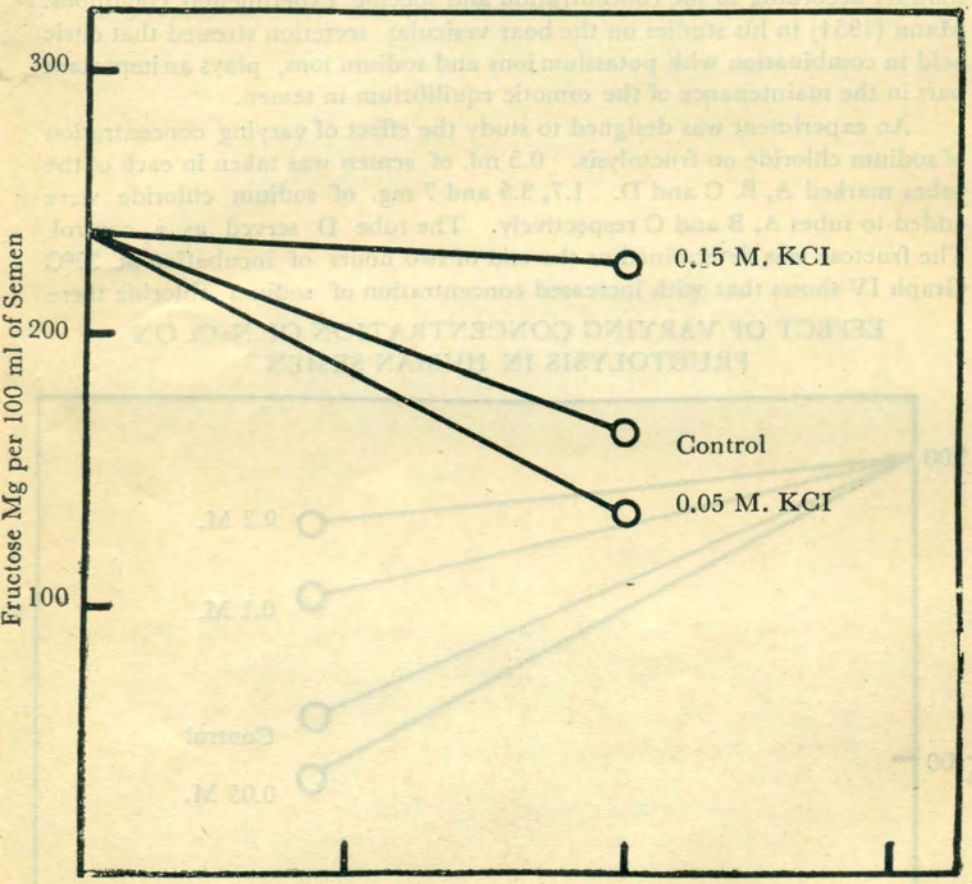


Graph IV

was greater inhibition of fructolysis, whereas low concentration actually increased fructolysis and motility. These results are in accordance with those of Neuport (1853).

The effect of 0 05 M. and 0.15 M potassium chloride was tried on fructolysis. Results are shown in Graph V. The higher concentration was found to inhibit fructolysis.

EFFECT OF KCI ON FRUCTOLYSIS IN HUMAN SEMEN



Graph V

Effect of Sodium Fluoride. Lardy and Phillips (1941) showed that 0.005 M fluoride was found to interfere with sperm motility and lactic acid production in bull.

A semen sample of count 130 million/ml. and motility 50 per cent was as a control. In both these samples fructose was estimated at the beginning of the experiment and then after two hours of incubation at 37°C. It was found that 1 ml. of untreated whole semen consumed in 2 hours, 45 per cent of the fructose initially present, whereas the fluoride treated sample utilised in the same period only 25 per cent fructose. The untreated spermatozoa were found to be perfectly motile, but those in the fluoride treated semen were mostly immotile.

Effect of Magnesium Sulphate. Lardy and Phillips (1943) have shown that magnesium has a beneficial effect on the motility of bull spermatozoa and to obtain optimum motility they advised the addition of atleast 0.012 M magnesium to diluted bull semen. The effect of magnesium on the fructolyis in human semen was determined as follows :

A 0.05 M magnesium sulphate was added to 1 ml. of semen sample having a count of 100 million/ml. of semen and motility of 55 per cent. A control was also incubated at 37°C. Table I shows the content of fructose present initially and after 2 hours. The fructose utilisation of magnesium sulphate treated spermatozoa was higher than the untreated ones.

TABLE I

Effect of Magnesium sulphate on Fructolysis in Human Semen.

Time	mg. Fructose/100 ml. semen control	mg. Fructose/100 ml. semen. $MgSO_4$ treated semen
0.5 hrs	350	350
2.5 hrs	260	220

DISCUSSION

Storage of semen in cold has assumed great importance in the preservation of spermatozoa in a healthy viable condition. Information is of vital importance for artificial insemination. Chang and Walton (1940) reported that cooling to a temperature just above 0°C was not harmful provided the cooling was done gradually. The temperature could be lowered by successive cooling. Sudden cooling of ejaculated semen produces the so called temperature shock which involves a rapid and irreversible loss of motility and fertilizing power. Mann and Lutwak-Mann (1955) reported that in rapid cooling of ram semen to 0°C., the semen was deprived of its fructolysing power whereas a slow cooling had practically no effect. One of the immediate

effects of sudden cooling was a decrease in the content of adenosine triphosphate. They observed that cytochrome was released during cold shock and so also some intracellular proteins. Oxygen uptake increased when succinate and cytochrome C were added to the suspension. Sheth and Rao (1959, b) observed that the succinic dehydrogenase activity of human semen went up in the cold. This in all probability is also due to the release of intracellular enzymes into the seminal plasma due to the temperature shock.

Our results on the effect of estrogens on the fructolysis of human spermatozoa is not in confirmity with the observation of Gassner and Hopwood (1955) who found that estradiol in concentration of 128 microgram per ml. of semen reduced fructolysis in bull spermatozoa. It has been observed by us that estrone in concentration of 50 microgram per ml. of semen stimulates fructolysis. This result is of particular importance since Rao, Hakim and Shahani (unpublished data) observed that human cervical mucus contains estrone. The secretion of mucus is known to increase during the ovulatory phase (Lamar, 1940, Abarbanel, 1946, and Bergman, 1959). Since cervical mucus contains estrogen and also our observations show that estrogen enhances fructolysis it can be inferred that the spermatozoa have a better chance of surviving for a longer period during the ovulatory phase of the menstrual cycle.

Our observation that progesterone inhibits fructolysis is in conformity with the observation of Lamar (1940) Abarbanel (1946) and Bergman (1959) that mucus from the post ovulatory phase or the progestational phase inhibits motility of spermatozoa. Testosterone in a concentration of 50 microgram per ml. of semen also shows a beneficial effect on fructolysis. These results are again contradictory to the results obtained by Gassner and Hopwood (1955) working with bull semen.

The effect of succinate and malonate on the fructolysis of human spermatozoa is of importance in the study of metabolism of human spermatozoa. MacLeod (1943) showed that in the presence of succinate human spermatozoa showed a higher rate of oxygen consumption, though there was not a simultaneous increase in motility. He actually observed a lower rate of glycolysis. Our results show that succinate in concentration of 0.05 M initially increases fructolysis and then on further incubation the rate of fructolysis falls down below the original rate.

Lardy and Phillips (1941) observed that malonate in concentration of 0.01 M inhibited the oxygen uptake of washed spermatozoa to the extent of 80 per cent but had no effect on motility. MacLeod observed that malonate in the same concentration inhibited methylene blue reduction in the presence of glucose. Our observations show that malonate in higher concentration (0.1 M) inhibits both fructolysis and motility of spermatozoa.

Spermatozoa treated with sodium fluoride were unable to utilise fructose as efficiently as the untreated spermatozoa. Mann and Lutwak-Mann (1948b) got the same results in their study with ram spermatozoa. They attribute the lowered rate of fructolysis to the inhibition of enolase.

Hepple and Hilmoe (1953) observed that magnesium has a powerful activating effect on adenosine triphosphatase of spermatozoa. Lardy and Phillips showed that 0.012 M magnesium improved the motility of bull sperm. Milavnov (1934) reported that sulphate ions have a beneficial effect on the spermatozoa by virtue of their protective effect on the lipid capsule of spermatozoa. This may be the reason why magnesium sulphate increases the fructolysis of human spermatozoa.

Blackshaw and Emmens (1951) in their studies with ram, bull and human spermatozoa showed that at all pH levels, hypertonic solutions were less harmful to motility than to hypotonic media, and that the relatively slight adverse effect of hypertonicity could be diminished by partial replacement of sodium chloride with glucose. Mann (1958) has studied the effect of hypertonic solutions of sodium chloride on the metabolism and found that fructolysis is abolished completely in the presence of 5 per cent sodium chloride. Recently, spermicidal activity of concentrated solutions of sodium chloride is made use of in the so called salt jellies. These have been investigated by Gamble (1953) as potential chemical contraceptives. Our studies show that at lower concentration of sodium chloride (0.05 M) fructolysis is activated, whereas in higher concentrations (0.2 M and above) it is inhibited.

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