Studies on the Metabolism of Semen

4. AEROBIC AND ANAEROBIC UTILIZATION OF FRUCTOSE BY SPERMATOZOA AND SEMINAL VESICLES

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Previous studies on the metabolism of semen have furnished evidence that the reducing carbohydrate in seminal plasma is fructose, which is readily converted by the spermatozoa to lactic acid, thus providing an important source of energy for the sperm cells (Mann, 1946*a*, *b*, *c*). The rate of fructolysis represents an accurate and at the same time simple means of evaluation of semen; the fructolysis index (mg. fructose utilized by 10^9 spermatozoa in 1 hr. at 37°) in normal bull and ram semen is 1·4–2·0, while semen with poor sperm motility gives much lower index values; azoospermic and necrospermic semen was shown to be altogether unable to metabolize fructose (Mann, 1948*a*, *b*). Fructose originates in the accessory glands of reproduction, mainly the seminal vesicles, but in some species it is found also in the ampullae and in certain parts of the prostate organ (Mann, 1946*c*; Davies & Mann, 1947). The process of fructose formation is initiated and controlled by the testicular hormone; a hormonal deficiency due, for example, to castration, causes invariably a decrease or disappearance of seminal fructose, but treatment with testosterone promptly restores the ability of the accessory glands to produce fructose (Mann & Parsons, 1947).

In this paper it will be demonstrated that fructolysis is a characteristic feature of both the aerobic and anaerobic metabolism of semen. Unlike spermatozoa, the seminal vesicles will be shown to lack the ability to utilize fructose anaerobically. Finally, the position will be discussed of fructolysis in relation to glucolysis and respiration, in both semen and seminal vesicles.

EXPERIMENTAL

Material. Ram semen was obtained by the method of Walton (1945); the procedure for washing the spermatozoa and the composition of the special Ringer solution were the same as previously described (Mann, 1945*a*, *b*, *c*). Seminal vesicles from fully fertile rats were slit open along the outer edge and washed in Ringer solution to remove the secretory fluid. Portions of the basal parts of the organ (8–10 mg., dry weight) were used so as to include as much as possible of the 'coagulating gland', which in rats contributes, together with the dorsal prostate, the bulk of seminal fructose. Seminal glands from bulls were collected from freshly slaughtered animals.

Methods. Three methods were used for the assay of fructolysis: (1) manometric estimation of acid production was carried out in Barcroft differential manometers with gas outlets, by measuring the CO_2 output using Ringer-bicarbonate and a gas mixture of 95 % N_2 and 5 % CO_2; (2) lactic acid was estimated by the method of Friedemann, Cotonio & Shaffer (1929); (3) fructose was determined colorimetrically as described before (Mann, 1948*a*, *b*). Respiration was measured manometrically in air and Ringer-phosphate.

RESULTS

Utilization of fructose by sperm

Anaerobic fructolysis. Certain points which emerged from the study of fructolysis in washed spermatozoa are illustrated in Fig. 1. The experiment recorded in this figure was carried out with suspensions of washed spermatozoa of ram in Ringer-bicarbonate solution, incubated anaerobically by shaking at 37° in Barcroft manometers filled with 95 % N_2 and 5 % CO_2. In each case the final volume of the mixture in the manometric flask was 2·5 ml. The following mixtures were used: I, $0{\cdot}65 \times 10^9$ cells + 1·9 mg. fructose; II, $0{\cdot}65 \times 10^9$ cells + 1·9 mg. glucose; III, $0{\cdot}13 \times 10^9$ cells + 1·9 mg. fructose. In I and II the acid formation followed the course of a straight line so long as there was still a little sugar left; after 110 min. incubation the final yield of CO_2 was in I: 420 μl. CO_2, corresponding to 1·68 mg. lactic acid; in II: 410 μl. CO_2, corresponding to 1·64 mg. lactic acid. By chemical analysis of lactic acid, 1·63 mg. was obtained in I, and 1·58 mg. in II. Estimation of sugar showed that only 0·05 mg. fructose was left in I, and 0·07 mg. glucose in II. Taking the 60 min. value as the basis for the calculation of the metabolic rate, one finds that 290 μl. CO_2 or 1·16 mg. lactic acid has been produced from fructose or glucose alike by $0{\cdot}65 \times 10^9$ sperm cells contained in mixtures I and II, i.e. 1·78 mg./10^9 sperm/hr. However, in III the acid amounted to not more than 24 μl. $CO_2/0{\cdot}13 \times 10^9$ sperm/hr., that is only 0·77 mg./10^9 sperm/hr.

Aerobic fructolysis and O_2 *consumption.* Aerobic experiments were carried out with the same ram semen as above, but the suspensions of washed spermatozoa contained phosphate instead of bicarbonate; the amounts of sugar added were the same as before. The O_2 uptake in suspensions I and II can be seen to follow a linear course throughout the period of 110 min. (Fig. 2). However, whereas in the course of anaerobic activity (Fig. 1), suspensions

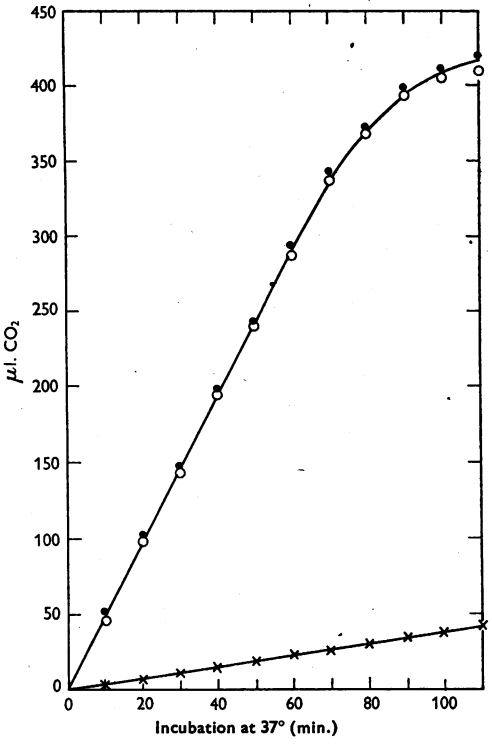

Fig. 1. Anaerobic fructolysis and glucolysis by washed spermatozoa. I: ●——● $0{\cdot}65 \times 10^9$ ram sperm + fructose; II: ○——○ $0{\cdot}65 \times 10^9$ ram sperm + glucose; III: ×——× $0{\cdot}13 \times 10^9$ ram sperm + fructose.

I and II used up nearly all the sugar, in the corresponding aerobic experiment (Fig. 2), out of 1·9 mg. sugar added, 0·42 mg. fructose and 0·48 mg. glucose, respectively, were found to be still intact after 110 min. In the same samples lactic acid was determined; 1·03 and 0·98 mg. lactic acid were found at the end of 110 min. respiration period in I and II respectively. It can be seen from Fig. 2 that the sperm suspensions I and II, each containing $0{\cdot}65 \times 10^9$ sperm/2·5 ml. Ringer solution, consumed 130 μl. O_2 in 1 hr. or 200 μl. $O_2/10^9$ sperm/hr. However, suspension III which is five times more diluted than I and II ($0{\cdot}13 \times 10^9$ sperm/2·5 ml. Ringer solution) consumed only 15 μl. O_2 or 115 μl. $O_2/10^9$ sperm/hr. The low rate of respiration in III recalls the previously mentioned low rate of anaerobic

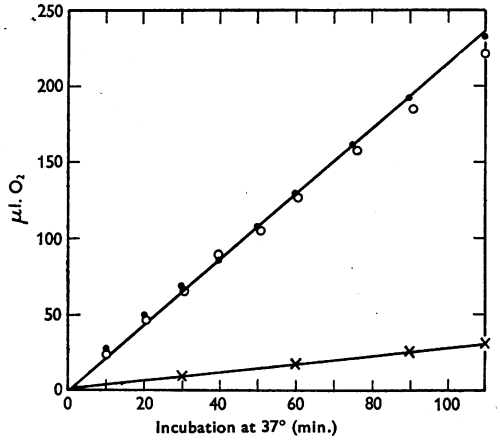

Fig. 2. Oxygen consumption of ram spermatozoa. I: ●——● $0{\cdot}65 \times 10^9$ ram sperm + fructose; II: ○——○ $0{\cdot}65 \times 10^9$ ram sperm + glucose; III: ×——× $0{\cdot}13 \times 10^9$ ram sperm + fructose.

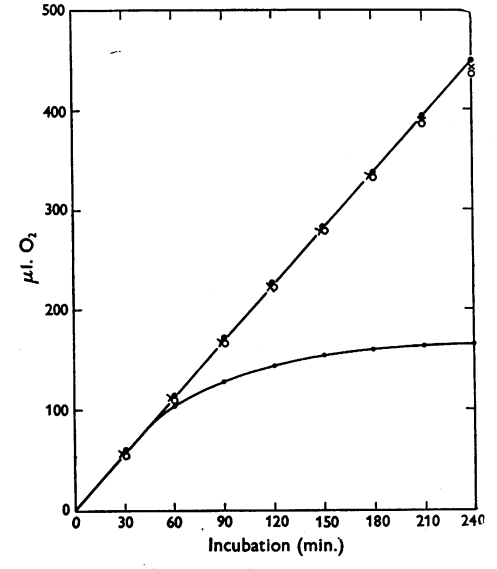

Fig. 3. Effect of fructose, glucose and lactate on the respiration of washed spermatozoa. 1 ml. ram semen diluted with 3 ml. Ringer solution, centrifuged, sperm washed with 5 ml. Ringer solution, resuspended in Ringer solution and diluted to 16 ml. Each manometer flask contained 2 ml. sperm suspension, corresponding to $0{\cdot}45 \times 10^9$ sperm cells, and 0·5 ml. isotonic phosphate buffer with: ·——· no additions; ●——● 1 mg. fructose; ○——○ 1 mg. glucose; ×——× 1 mg. lactic acid (as Na lactate).

glycolysis and must be attributed to the same cause: the injurious effect of dilution on spermatozoa.

It has been known that ram spermatozoa freed from seminal plasma, and thus deprived of glycolyzable material, retain their ability to consume oxygen at an almost normal rate of respiration of about 200 μl. $O_2/10^9$ sperm/hr. (Lardy, Winchester & Phillips, 1945; Mann, 1945 *c*). Thus the aerobic metabolism in spermatozoa appeared independent of fructolysis. However, in the present study, with suitable dilutions of washed spermatozoa, it was found that the O_2 uptake in such suspensions remained constant only for a limited period of time,

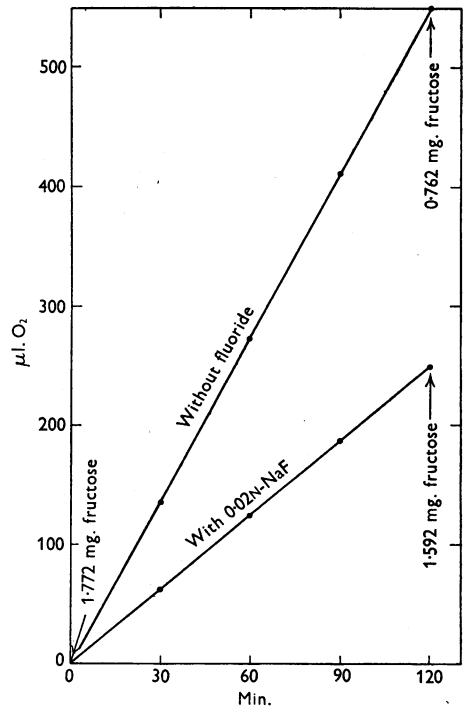

Fig. 4. Effect of fluoride on the respiration and aerobic fructolysis of ram semen. Each manometer flask contained 0·5 ml. whole semen diluted with 1·5 ml. Ringer-phosphate solution.

and that during the later stage it declined progressively unless the sperm were provided with an additional source of oxidizable material such as fructose, glucose or lactate. Their effect on the O_2 uptake of washed spermatozoa is illustrated by Fig. 3, whence it can be seen that all three of them maintained equally well the initial rate of respiration for a considerable length of time, although they did not raise significantly the initial rate of O_2 consumption. On the other hand, if added to a respiring suspension of washed spermatozoa at a later stage, when the respiration had already begun to decline, they prevented further deterioration in the rate of O_2 consumption.

In order to explore the significance of glycolysis in the aerobic metabolism and survival of spermatozoa, use was made of certain substances such as iodoacetate and fluoride which were previously shown (Lardy & Phillips, 1943) to interfere with sperm activity. Iodoacetate proved to be a rather strong inhibitor not only of fructolysis but of the sperm respiration as well. More interesting results were achieved with fluoride. It was possible, using a suitable concentration of NaF, to suppress the fructolytic activity of whole semen much more strongly than the respiration. This can be seen from Fig. 4, which shows the O_2 uptake of two identical samples of semen, one treated with fluoride, while the other served as a control. In both these samples fructose was estimated at the beginning of the experiment and then after 2 hr. aerobic incubation in Barcroft manometers at 37°. The results of fructose analyses are indicated by arrows on Fig. 4. It can be calculated from these values that 0·5 ml. untreated whole semen consumed in 2 hr. 550 μl. O_2 and 1·01 mg. fructose, whereas the corresponding fluoride-treated sample utilized in the same period 250 μl. O_2, but only 0·18 mg. fructose. When at the end of a 2 hr. period the motility of the sperm was examined, the untreated spermatozoa were found to be perfectly motile, but those in the fluoride-treated semen were mostly immotile.

Fructose and glucose utilization in the seminal vesicle

It was shown in the foregoing that spermatozoa are cells capable of utilizing fructose and glucose both anaerobically and aerobically. In this respect they differ from most other animal tissues; the latter are able to metabolize fructose aerobically, but seldom show themselves capable of metabolizing it anaerobically at a rate comparable to that of glucose (Dickens & Greville, 1932, 1933). A study of carbohydrate metabolism in the seminal vesicle of the rat gave the following results, illustrated in Fig. 5. The anaerobic acid production in the seminal vesicles was found to be equally low in the absence as in the presence of added fructose. However, when glucose was added instead of fructose, it increased very strongly the rate of anaerobic glycolysis in seminal vesicles. It should be pointed out, however, that even the increased rate of glycolysis, as observed in the seminal vesicle in presence of glucose, was below the rate of glycolysis of spermatozoa. Assuming that the average rate of glycolysis in semen is 1·7 mg. sugar or 425 μl. $CO_2/10^9$ sperm/hr. and that the dry weight of 10^9 sperm is 30 mg., we arrive at the value of $Q_L^{N_2} = +14{\cdot}2$ for the glycolysis quotient of spermatozoa. The seminal vesicle tissue, on the other hand, has been shown to produce anaerobically 2·3 μl. CO_2/mg. dry wt./hr. and thus to have a quotient $Q_L^{N_2} = +2{\cdot}3$.

In view of the marked differences in anaerobic metabolism which exist between the sperm cells and seminal vesicles the possibility was considered that similar differences may also prevail under aerobic conditions. Seminal-vesicle slices incubated aerobically respired much better in presence of additional sugar, and the increase brought about by fructose was, if anything, even higher than that due to glucose (Fig. 6). The Q_{O_2} values for rat seminal-vesicle slices, calculated from the data of Fig. 6, are $-5{\cdot}3$ in presence of fructose, $-4{\cdot}2$ in presence of glucose, and $-2{\cdot}0$ in absence of added sugar. Even the highest of the three values is far below the Q_{O_2} of washed spermatozoa. Taking Fig. 3 as the basis of calculations, the O_2 uptake of ram sperm amounts to 112 μl. $O_2/0{\cdot}45 \times 10^9$ sperm/hr., i.e. 250 μl. $O_2/10^9$ sperm/hr. The dry weight of 10^9 sperm cells represents some 30 mg. so that the Q_{O_2} of spermatozoa is $-8{\cdot}3$.

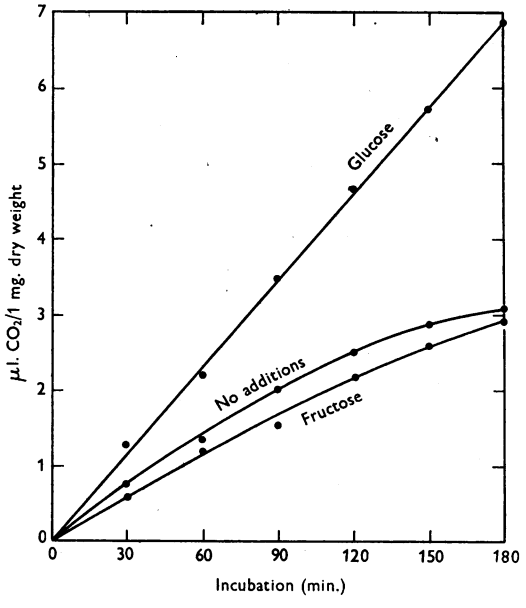

Fig. 5. Anaerobic fructolysis and glucolysis in rat seminal vesicle.

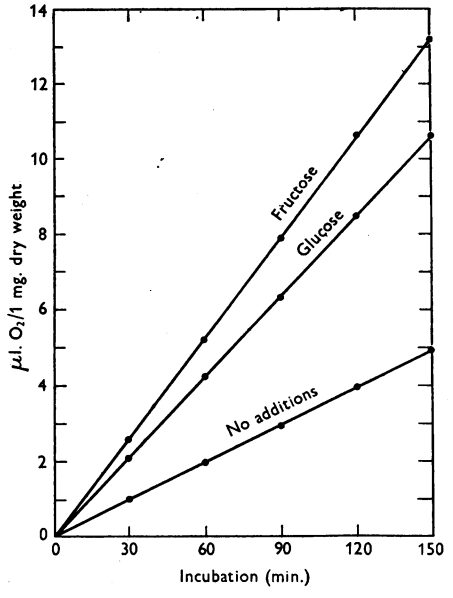

Fig. 6. Respiration of rat seminal vesicle in presence of fructose and glucose.

Bull seminal glands were investigated in the following manner: the fresh gland was divided into two symmetrical portions, in one of which fructose was estimated at once, while the other was incubated at 37° for 2 hr. The level of fructose in the incubated sample did not differ markedly from the control. Similar results were obtained when the glands were minced or extracted with water and the pulp or aqueous extract used for incubation. A few experiments were also carried out in which minced seminal glands, slices or extracts, were incubated in presence of added carbohydrates. In presence of glucose, aerobically, a formation of free fructose was observed in slices, but the quantities were small. On the other hand, considerable quantities of 6-phosphofructose, together with 6-phosphoglucose, were formed from glycogen and Cori ester, by dialyzed and non-dialyzed extracts; but there was no increase in free fructose or lactic acid. It was also found that when dialyzed extract prepared from seminal vesicles was added to an actively glycolyzing Meyerhof muscle extract, it checked the formation of lactic acid from glycogen and led to the accumulation of 6-phosphohexose esters. It should be pointed out, however, that the extracts from seminal glands contain very active phosphatases which dephosphorylate adenylic acid, adenosine triphosphate and cozymase. Thus it is probable that the effects described above are at any rate partly due to the inactivation of the glycolytic coenzymes.

DISCUSSION

In bull and ram semen the anaerobic survival of spermatozoa is closely dependent upon the presence of fructose. If ram or bull spermatozoa are washed free from seminal plasma, and thus deprived of fructose, they soon become immotile under anaerobic conditions. But their survival can be extended considerably by the addition of glycolyzable sugar. So far, there is no evidence which would point to the existence of other anaerobic processes in semen,

capable of supplying the spermatozoa with metabolic energy on a scale comparable with fructolysis. Under aerobic conditions, on the other hand, spermatozoa can survive temporarily, even after the removal of seminal plasma. However, our experiments on washed ram spermatozoa showed that their ability to take up oxygen is of relatively short duration, but that it can be maintained for a considerable length of time by the addition of certain substances. Among these are fructose and lactate, both of which are normally present in semen. These findings lead to the conclusion that the metabolism of fructose plays an important role not only in the absence of oxygen but also in aerobic conditions. It remains for further study to ascertain precisely what type of metabolism, aerobic or anaerobic, predominates in spermatozoa during their existence in either the male or female genital tract. However, so far as *in vitro* storage is concerned, such as, for instance, for the purpose of artificial insemination, there is little doubt that the predominant process which supplies the vital energy to the cells is fructolysis and not respiration (Mann, 1948 *a*, *b*).

Although fructose is the 'physiological' carbohydrate, and there is practically no glucose in whole semen, yet spermatozoa which have been washed free from seminal fructose are capable of utilizing added glucose to the same extent as fructose. This behaviour of spermatozoa is in striking contrast to that of most other animal tissues, including the seminal vesicles which in most species produce the bulk of seminal fructose. This tissue utilizes glucose anaerobically, but not fructose. Aerobically, however, the seminal vesicles can utilize both glucose and fructose, and in this respect they do not differ from spermatozoa.

SUMMARY

1. The process of fructolysis plays an essential role in the survival of mammalian spermatozoa both under anaerobic and aerobic conditions. Anaerobic fructolysis provides the main source of energy for spermatozoa. Aerobically both fructose and lactic acid function by prolonging and maintaining the sperm respiration.

2. In buffered sperm suspensions fructolysis continues at a steady rate, and follows a linear course until practically the entire sugar content has been exhausted. The rate of fructolysis is greater under anaerobic than aerobic conditions. Excessive dilution of sperm suspensions causes a decrease in the rate of both fructolysis and respiration.

3. By using a suitably chosen concentration of sodium fluoride it is possible to abolish fructolysis completely while the respiration is only partly suppressed. Under such conditions it was found that the spermatozoa were immotile even although there was still some O_2 consumption.

4. Spermatozoa washed free from fructose-containing seminal plasma can utilize anaerobically to the same extent added fructose or glucose. In this respect the sperm cells differ from the fructose-producing tissue, the seminal vesicle, which utilizes glucose anaerobically, but not fructose. Aerobically, however, both sperm and the seminal vesicles are capable of metabolizing both fructose and glucose.

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