THE METABOLIC ACTIVITY OF THE BOVINE EPIDIDYMIS

I. UTILIZATION OF GLUCOSE AND FRUCTOSE

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Summary. The epididymides of five mature bulls were unilaterally isolated by ligation at the efferent ductules. After 1 month's delay and electroejaculation to ensure sperm clearance of the isolated epididymis, the epididymides were removed, divided into five segments and subjected to a radio-respirometric study. The metabolism of [1- ^{14}C]glucose (G-1), [2- ^{14}C]glucose (G-2), [3,4- ^{14}C]glucose (G-3,4), [6- ^{14}C]glucose (G-6), and uniformly labelled glucose (G-U) and fructose (F-U) by normal and spermatozoa-free bovine epididymal tissue *in vitro* was determined.

The patterns of $^{14}\text{CO}_2$ evolution were consistent with the Embden-Meyerhof and Krebs' cycle pathways of glucose metabolism, but utilization of G-1 exceeded G-6 throughout all zones. The mean G-1:G-6 ratio was 3.4 in control and treatment tissues which indicated a high rate of pentose-cycle activity. There was a significantly higher utilization of all substances by the control tissues over the treatment tissues. The F-U was utilized less than any of the labelled glucose substrates.

Zone 3 (corpus epididymidis) of treatment and control tissues used substrates at a higher rate than any other zone. Zone 3 was also noted for its high rate of pentose-cycle activity giving rise to speculation about possible synthetic activity. Zone 5 (cauda epididymidis) was the least metabolically active of all the zones studied.

INTRODUCTION

While the epididymis has been the subject of scientific investigation for some time, much of this early research appears to be contradictory or inconclusive (Maneely, 1959). Later studies have refined much of this early work and further outlined the mechanisms involved (Crabo, 1965; Orgebin-Crist, 1969; Waites & Setchell, 1969).

Epididymal histology and cytology (Nicander, 1957, 1958; Blackshaw & Samsoni, 1964), chemical components of epididymal fluid (Scott, Wales, Wallace & White, 1963; Crabo, 1965), and tubule secretion and resorption of fluid (Waites & Setchell, 1969; Barker & Amann, 1971) have been studied. Alterations of the sperm cell during passage through the epididymis has also

been studied (reviewed by Orgebin-Crist, 1969). Metabolism of epididymal spermatozoa has been investigated (Wu, McKenzie, Fang & Butts, 1959; Murdoch & White, 1968; Voglmayr, Larsen & White, 1970), but the metabolism of the epididymal tubule has received little attention.

Annison, Scott & Waites (1963) studied the oxidative metabolism of glucose and acetate in the testicular and epididymal tissue of the anaesthetized ram. These investigations showed that glucose was a more important metabolite than acetate. In a similar study, Setchell & Waites (1964) confirmed the importance of glucose as a metabolite. In an *in-vitro* study, Elliot (1965a) showed that epididymal metabolism of the rat was glycolytic in nature. In a subsequent paper, Elliot (1965b) showed that efferentiectomy lowered the level of both aerobic and anaerobic glycolytic activity of epididymal tissue.

Johnson & Turner (1971) reported high pentose-cycle activity of the tissues from the testis, caput epididymidis and cauda epididymidis in the rat, rabbit and mouse. Using the same species, Kraft & Johnson (1972) showed extensive pentose-cycle activity in the epididymis. Their study also showed patterns of $^{14}\text{CO}_2$ recovery from specifically labelled glucose substrates that were characteristic of the Embden-Meyerhof and Krebs' cycle pathways of glucose metabolism. Fructose was generally a less important metabolite than glucose in the epididymides of all the species studied.

The purpose of this study was to investigate further the metabolic activity of tissues obtained from the five zones of the normal and efferentiectomized bovine epididymis. A radio-respirometric procedure was used to measure the metabolism *in vitro* of glucose and fructose by these tissues.

MATERIALS AND METHODS

In this study, the epididymides of five mature bulls were utilized. The efferent ductules were unilaterally ligated leaving the contralateral epididymis as control tissue. The treatment (ligated) side was randomized among bulls. Surgical procedure was carried out employing a modification of the procedure previously used on the ram (Voglmayr, Scott, Setchell & Waites, 1967) in which one epididymis of each bull was isolated from the testicular contribution. An incision approximately 8 cm long was made on the anterior surface of the scrotum over the area of the caput epididymidis. Upon exposure of the tunica vaginalis, a similar incision was made, care being taken to avoid incising the tunica albuginea. Proper placement of the initial incision and use of Weitlaner retractors ensured favourable exposure of the caput epididymidis. An incision was made into the connective tissue border of the caput and tunica albuginea. This incision was continued upward under the epididymis between the caput and the tunica albuginea. When a small flap of the caput was released from the testis in this manner, Allis forceps were used to lift the caput away from the testis improving exposure to the operating area. Cutting with a scalpel or scissors along the border of the epididymis and using blunt dissection in the area under it, the head was gradually lifted from the testis until the efferent ducts were exposed.

The efferent ducts were ligated taking care to avoid interrupting the vasculature of the area. Any trauma in this area caused excessive haemorrhage which

was difficult to arrest. The ligation was made as close to the entry of the efferent ducts into the caput as possible. Transecting the efferent ducts between the testis and the ligation left the epididymis isolated from the testis while the testis was allowed to drain into the peritoneal cavity of the scrotum.

An absorbable gelatin sponge pad was inserted under the caput to halt any haemorrhage that had occurred. Ensuring that the epididymal tubule was neither punctured nor occluded, two stitches were taken in the connective tissue border of the caput and the tunica albuginea to retain the caput in its proper position.

TEXT-FIG. 1. Schematic drawing of bull testis and epididymis showing Nicander's (1958) zones (a) in relation to zones of the present study (b).

The incision was then flushed with sterile physiological saline (37° C) and treated with nitrofurazone. The incised tunica vaginalis was closed over the epididymis with size 0 suture material. The incision of the scrotum was treated with nitrofurazone and closed using 16-mm stainless steel wound clips situated approximately 1 cm apart. A bacitracin-neomycin topical ointment (Myce-tracin, Upjohn) was applied to the closed wound to promote healing.

Following surgery, and once daily for 5 days thereafter, 5 ml of a penicillin-

dihydrostreptomycin suspension (Combiotic, Pfizer) was administered intramuscularly.

Approximately 1 month after surgery, electroejaculates were collected from all bulls every day for 3 consecutive days to ensure that the ligated epididymides were free of spermatozoa. Following this, the testes and epididymides were removed. Immediately after castration, the epididymis was removed and divided into five zones (Text-fig. 1). This segmentation was necessary in order to sample tissue consistently from the caput, corpus and cauda plus one additional zone each of the caput and cauda which appeared different due to tubule size or coloration.

Each segment of the epididymis was placed in a watch-glass containing Krebs-Ringer bicarbonate (KRB) maintained at 4° C and stripped of its connective tissue covering plus connective tissue within the zone, as far as possible. The connective tissue was removed from all segments and incubation begun within 1½ hr following castration. During this time, the tissue was always maintained at approximately 4° C. Then, using a method described earlier by Johnson & Turner (1971) and Kraft & Johnson (1972), 3 to 5 mg of tissue were weighed to the nearest 0.2 mg and placed in a 0.5-ml ampoule containing the following substances: 0.05 ml calcium-free KRB containing 0.15 µmol of twenty principal amino acids, 10 i.u. penicillin and 0.5 µCi of ¹⁴C-labelled substrate. The substrates used and specific activities were: [1-¹⁴C]glucose (G-1), 47.8 µCi/µmol; [2-¹⁴C]glucose (G-2), 58.8 µCi/µmol; [3,4-¹⁴C]glucose (G-3,4), 13.8 µCi/µmol; [6-¹⁴C]glucose (G-6), 44.9 µCi/µmol; [U-¹⁴C]glucose (G-U), 207.0 µCi/µmol; and [U-¹⁴C]fructose (F-U), 121.0 µCi/µmol. A weighed tissue sample from each zone was placed in an ampoule containing each of these substrates. After the tissue was placed in the ampoule, the sample was aerated with a gas mixture (3% CO₂, 20% O₂, 77% N₂) for 30 sec.

The ampoule was attached to a CO₂ trap by an elbow connector fabricated from polyethylene tubing (Tygon). The CO₂ trap, a glass bulb, was made by breaking a 6-mm diameter glass rod into 20-mm segments and heat-sealing one end. A piece of filter paper was inserted into the glass bulb. Before insertion into the elbow connector, 0.1 ml diethanolamine buffer was applied to the filter paper.

This closed system (ampoule, connector, CO₂ trap) was placed in an incubator at 35° C. The CO₂ traps were replaced hourly for 5 hr. Upon removal of

EXPLANATION OF PLATE 1

Histology of control and treatment bull epididymides (Zones 1 to 3). All tissues were preserved in Bouin's fixative and stained by haematoxylin-eosin. × 78.

FIG. 1. Zone 1 of control epididymis. Note tubule size, thickness of tubule wall and sperm concentration.

FIG. 2. Zone 1 of treatment epididymis. Note absence of spermatozoa and decrease in tubule size.

FIG. 3. Zone 2 of control epididymis. Note apparent increase in sperm concentration, decrease in thickness of tubule wall, and increase in staining of nuclei.

FIG. 4. Zone 2 of treatment epididymis. The tubule has decreased in size and epithelial cells are present in lumen. Note absence of spermatozoa.

FIG. 5. Zone 3 of control epididymis. Note epithelial height. Nuclei are confined to the basal area of the tubule. Sperm presence is evident.

FIG. 6. Zone 3 of treatment epididymis. Epithelial height has decreased and nuclei are no longer held so closely to basal area. Note absence of spermatozoa.

PLATE I

(Facing p. 204)

PLATE 2

Histology of control and treatment bull epididymides (Zones 4 and 5). Tissues were preserved and stained as in Plate 1. $\times 78$.

FIG. 1. Zone 4 of control epididymis. Note increase in sperm concentration and diffuse location of nuclei.

FIG. 2. Zone 4 of treatment epididymis. Note decrease in tubule size, decrease in thickness of tubule wall, and convolution of tubule wall. Spermatozoa are absent.

FIG. 3. Zone 5 of control epididymis. Note increase in tubule size, decrease in epithelial height, and increase in sperm concentration.

FIG. 4. Zone 5 of treatment epididymis. Note absence of spermatozoa but presence of epithelial cells in the lumen.

(Facing p. 205)

the fifth CO_2 trap, 0.1 ml of 1 N- H_2SO_4 was added to the ampoule to halt further metabolism and release any residual CO_2 from the sample. The system was again closed for 30 min before the sixth and final trap was removed.

As the CO_2 traps were removed, they were placed into vials containing 10 ml scintillation fluid. The samples were refrigerated overnight before counting in a refrigerated scintillation spectrophotometer (Tricarb, Packard Model 3380). The counts/min/mg tissue were converted to disassociations/min/mg tissue. Picomoles of substrate utilized/mg tissue were calculated and comparisons made of substrate utilization by tissues from all five zones of the treatment and control epididymides.

During the tissue sampling procedure, tissue segments from each zone of each epididymis were placed into vials containing Bouin's fixative solution in preparation for making tissue mounts. Slides were made from these mounts stained with haematoxylin-eosin and examined under the microscope.

The data resulting from this study were subjected to least squares analysis of variance followed by Duncan's Multiple Range Test to determine points of significant difference.

RESULTS

Histological samples (Plates 1 and 2) show the presence and absence of spermatozoa in the control and treatment tissues, respectively. The tubules of the

TEXT-FIG. 2. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose compounds and uniformly labelled glucose and fructose by Zone 1 of the control bull epididymides.

treatment epididymides (Pl. 1, Figs 2, 4 and 6; Pl. 2, Figs 2 and 4) regressed in size, apparently due to lack of testicular contribution.

Text-figures 2 to 11 show the hourly recovery rates of $^{14}\text{CO}_2$ from six different specifically labelled substrates within the five zones studied for both the control and treatment tissues. The general relationship of $^{14}\text{CO}_2$ recovery from each of the substrates in both the control (Text-figs 2 to 6) and treatment (Text-figs 7 to 11) tissues was as follows: G-3,4 > G-1 > G-2 > G-U > G-6 > F-U. Within zones, hours, and treatments, G-3,4 was always utilized preferentially ($P < 0.05$) and F-U utilized the least ($P < 0.05$) when compared to all other substrates.

TEXT-FIG. 3. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 2 of the control bull epididymides. Note change in scale expansion.

Comparison of the graphs reflects the fact that the control tissues (Text-figs 2 to 6) consistently yielded higher $^{14}\text{CO}_2$ recovery rates from all the substrates utilized ($P < 0.01$) than did the sperm-free tissues (Text-figs 7 to 11). There was a significant difference in preferential use of substrates by different zones for all substrates with the exception of G-2. The labelled substrates, G-U, G-1 and F-U ($P < 0.01$) and G-4 and G-6 ($P < 0.05$) all showed different utilization by zones. Substrates G-U, G-1 and G-6 were all utilized more ($P < 0.05$) by Zone 3 (Text-figs 3 and 9) than any other zone. Another trend showed $^{14}\text{CO}_2$ evolution from G-U, G-1, G-6 and F-U to be significantly less ($P < 0.05$) in Zone 5 (Text-figs 6 and 11) than in any other zone.

Text-figures 2 to 11 also demonstrate that there was a significant difference ($P < 0.01$) of $^{14}\text{CO}_2$ recovery between hours for all substrates. This difference was shown to be due only to the increased $^{14}\text{CO}_2$ production during the residual collection ($P < 0.05$) except for the first hourly collection of G-1 and G-6 which was significantly lower in $^{14}\text{CO}_2$ recovery ($P < 0.05$).

Within hours, there was also shown to be a statistical difference ($P < 0.01$) between the control and treatment tissues for all hours, $^{14}\text{CO}_2$ -evolution rates always being greater for the control tissues. Compared within hours, there was

TEXT-FIG. 4. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 3 of the control bull epididymides. Note reduction in scale expansion.

also a statistical difference ($P < 0.01$) between zones. Zone 1 (Text-figs 2 and 7) was higher than all other zones in $^{14}\text{CO}_2$ production every hour except the 5th hour and residual collection.

Within hours, there was a statistical difference ($P < 0.01$) in utilization of substrates by the tissues. Substrate G-3,4 was utilized preferentially in every zone over all other substrates during every hour; G-1 was significantly higher in the 4th and 5th hour; F-U was utilized at a lower rate than G-1 and G-3,4 during every hour ($P < 0.05$).

An estimation of pentose-cycle activity was given by determining G-1:G-6 $^{14}\text{CO}_2$ -evolution ratios for the first 2 hr of incubation. In the treatment tissues, Zones 1 to 5, the values were 1.8, 7.5, 2.8, 1.8 and 3.0, respectively. The corresponding values in the control tissues were 2.2, 2.0, 5.3, 3.7 and 3.8.

TEXT-FIG. 5. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 4 of the control bull epididymides.

TEXT-FIG. 6. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 5 of the control bull epididymides.

TEXT-FIG. 7. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 1 of the treatment bull epididymides. Note decrease in overall metabolism compared to control tissue.

TEXT-FIG. 8. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 2 of the treatment bull epididymides.

TEXT-FIG. 9. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 3 of the treatment bull epididymides.

TEXT-FIG. 10. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 4 of the treatment bull epididymides. Note increase in scale expansion.

TEXT-FIG. 11. Hourly and residual (Res.) $^{14}\text{CO}_2$ evolution from specifically labelled glucose and uniformly labelled glucose and fructose by Zone 5 of the treatment bull epididymis. Notice further increase in scale expansion.

DISCUSSION

The results from this study show significant differences in the metabolic rate between the control and treatment tissues. The spermatozoa-free tissue of the treated epididymis had a consistently lower metabolic rate than the normal, or control, epididymal tissue. This is supported by the work of Elliot (1965b) who found that efferentectomy decreased respiratory activity and glycolytic activity in the epididymal tissue of the mouse. He reported the decrease in respiratory activity to be due to absence of spermatozoa, and decrease in glycolytic activity to be due to metabolic changes in the epididymal epithelium. This difference in metabolic rate, noted in all zones, was demonstrated by the control: treatment ratios of $^{14}\text{CO}_2$ evolution for 'cauda epididymidis' tissues in the 5th hour of incubation. These values for G-U, G-1, G-2, G-3,4, G-6 and F-U were 3.5, 1.7, 2.4, 1.6, 2.9 and 3.1, respectively.

The relative recovery rates in this study for both control and treatment tissues were $\text{G-3,4} > \text{G-1} > \text{G-2} > \text{G-U} > \text{G-6} > \text{F-U}$. This relationship is consistent with Embden-Meyerhof glycolysis and the Krebs' cycle pathway as a major contributor to glucose metabolism. This relationship of $^{14}\text{CO}_2$ evolution corresponds to the pattern found by Free & VanDemark (1969) for rat, rabbit and chicken testicular tissue and by Kraft & Johnson (1972) in rat, rabbit and mouse epididymis, except for the preference of G-1 over G-2 due possibly to the high

degree of pentose-cycle activity in this present study. Pentose-cycle activity was indicated by high $^{14}\text{CO}_2$ evolution of G-1 compared to G-6 and persisted throughout the entire incubation period, as previously reported for the rabbit (Free & VanDemark, 1969) but not for the rat and chicken in the same study. Considering only the first 2 hr of the study, the average G-1:G-6 ratios were 3.4 with no difference between treatment and control tissues. These values represent a higher rate of pentose-cycle activity in the bovine epididymis than Johnson & Turner (1971) or Kraft & Johnson (1972) found in the mouse, rabbit or rat. They are also higher than rates reported in the ram testis by Murdoch & White (1968) or in the rat, rabbit and chicken testis by Free & VanDemark (1969). The similarity of the control and treatment G-1:G-6 ratios in this study supports the statement of Kraft & Johnson (1972) that the pentose-cycle activity found in the epididymis is presumably due to the metabolism of the tissue rather than the sperm cells contained therein.

Between zones, the only trends established by this study were that Zone 3 utilized three of the substrates at a significantly higher rate ($P < 0.05$) than all other zones. In Zone 5, the tissue utilized significantly less of four substrates. Despite the histological and cytological differences in the epididymal epithelium that exists between zones (Nicander, 1958), no distinct, overall fluctuations could be detected in the glucose metabolism of the five zones studied, with the exception of the previously mentioned pentose-cycle activity. The G-1:G-6 ratios were especially high in Zone 2 of the treatment tissue and Zone 3 of the control tissue. This shift in area of high activity cannot be explained at present; however, if these results reflect conditions *in vivo*, perhaps one or both of these zones hold special importance in their NADPH production, lipogenesis, or nucleic acid synthesis. It is interesting to note that in this study, Zone 3 utilized half of the substrates at a high rate and was high in pentose-cycle activity. Crabo (1965) showed that several electrolytes and glycerylphosphorylcholine increase in Zone 3 and Waites & Setchell (1969) indicated that a higher blood flow occurs in this region as compared to adjacent areas of the head and tail. Any or all of these could be of importance in the final maturation process of the sperm cell which is said (Orgebin-Crist, 1969) to occur in the area of the 'mid-corpus'. This region is included within Zone 3 of the present study.

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