ENERGY METABOLISM OF THE NORMAL AND CRYPTORCHID RAT TESTIS

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Summary. The concentrations of glycogen, glucose, ATP, glucose-6-phosphate and lactate were studied in normal and cryptorchid rat testes using enzymatic pyridine nucleotide methods. With the exception of ATP, all the substrates were significantly higher in the cryptorchid testes. The decrease of the energy metabolites and the accumulation of lactate were also studied in testes incubated in the absence of O₂ at the temperatures of 33.6° C and 36.6° C. The metabolic rate of the incubated organs was estimated in terms of the rate of use of high energy phosphate ($\sim$ P) calculated from changes in ATP, glucose and glycogen. A good parallelism between hexose utilization and lactate production was observed in both normal and cryptorchid testes. A rapid mobilization of glycogen, suggesting activation of phosphorylase, was observed in incubated cryptorchid testes. The calculated oxygen consumption of cryptorchid testes was about 100% higher than that of the normal testes at the same temperature. It is suggested that the basic metabolic rate of the interstitial tissue is higher than that of the seminiferous tubules.

INTRODUCTION

The present knowledge of the energy metabolism of the rat testis is limited and is based mainly on the results of studies *in vitro* on testicular slices and homogenates. Such studies have suggested that glucose is the major fuel of the testis (Himwich & Nahum, 1929; Dickens & Simer, 1930, 1931), and that there exists an active pentose phosphate cycle (Bloom, 1955; Field, Pastan, Herring & Johnson, 1960; Free, Cruz, Johnson & Gomes, 1968).

Experiments *in vivo* on the oxidative metabolism of the testis have been performed mainly on larger animals, especially on the ram (Annison, Scott & Waites, 1963; Setchell & Waites, 1964; Setchell & Hinks, 1967) but no relevant work has been published on the rat testis.

Both biochemical (Rudolph & Olsen, 1956; Schor, Cara & Perez, 1963) and histochemical (Niemi & Ikonen, 1962; Kormano, Härkönen & Kontinen, 1964) methods have been used to study the enzymes which function in known pathways of glucose metabolism and there is evidence on the basis of these findings that the seminiferous tubules and the interstitial tissue have a

qualitatively different type of metabolism. Metabolic studies on testes with atrophied tubules have led to a similar conclusion (Tepperman, Tepperman & Dick, 1949; Paul, Paul, Kopko, Bender & Everett, 1953; Steinberger & Wagner, 1961), but little attention has been paid to the substrates of the energy metabolism in normal or atrophied testes.

The testes will maintain function for a very short time through utilization of endogenous energy reserves, when incubated under N_2 in a closed system. In the absence of oxygen, this reserve has three major components: glycogen, glucose and ATP. Determination of these substrates offers an opportunity for studying the testicular energy metabolism of a small animal like the rat. Using testes with normal and atrophied tubules, differences in the metabolism of the tubules and the interstitial tissue will influence the result and give evidence of the metabolic characteristics of these two tissues. In the present study, measurements have been made of the major energy-yielding metabolites in the normal and experimentally cryptorchid rat testis.

EXPERIMENTAL

Animals

Adult, male, white rats of the Sprague-Dawley strain were used. For normal material, testes of thirteen 6-month-old rats were used. Ten rats were rendered cryptorchid at the age of $2\frac{1}{2}$ months by displacement of both testes into the abdominal cavity, invagination of the processus vaginalis and closure of the deep inguinal ring. These animals were studied 6 weeks later, together with twelve controls.

Preparation of material

Thirty minutes after injection of pentobarbitone (Nembutal, Abbot, 50 mg/kg), the testes were exposed through a midline incision in the lower abdomen, rapidly excised and frozen in Freon 12 (CCl_2F_2) chilled to the freezing point ($-150^\circ C$) with liquid nitrogen.

In incubation experiments, the excised normal testes were incubated either at $36.6^\circ C$ (inguinal temperature) or $33.6^\circ C$ (rat normal testicular temperature; Kormano, 1967) for various intervals in mineral oil through which N_2 was bubbled. Cryptorchid testes were incubated only at $36.6^\circ C$.

The time interval between the excision of the organ and freezing or beginning of incubation was 2 to 3 sec.

Analytical methods

The frozen samples were stored at $-75^\circ C$ until ready for analysis. Acid extracts were prepared by adaptation of the procedure of Nelson, Lowry & Passonneau (1966) from approximately 100 mg of tissue weighed in a cold room at $-20^\circ C$. A detailed description of the procedure has been given in an earlier paper (Härkönen, Palkama & Laasonen, 1968). Glycogen was measured from separate samples after alkaline digestion and ethanol precipitation (Good, Kramer & Somogyi, 1933). The glycogen, glucose, glucose-6-phosphate, ATP and lactate were determined with enzymatic pyridine nucleotide methods as

previously described by Lowry, Passonneau, Hasselberger & Schulz (1964), with minor modifications (Härkönen *et al.*, 1968). In a statistical analysis of the results, the *t* test was employed according to the method of Jonge (1964).

RESULTS

Normal values

The concentrations of glycogen, glucose and lactate were rather low when compared with some other tissues (Härkönen, Passonneau & Lowry, 1969). The concentration of ATP was relatively high. The concentrations of metabolites and calculated high energy phosphate ($\sim P$) in the testes of different experimental groups are presented in Table 1. With the exception of ATP, all

TABLE 1
THE CONCENTRATION OF VARIOUS SUBSTRATES OF THE ENERGY
METABOLISM IN NORMAL AND CRYPTORCHID TESTES

Metabolite	Normal testis* mmol/kg wet wt	Cryptorchid testis† mmol/kg wet wt	
	Mean $\pm$ S.E.	Mean $\pm$ S.E.	P <
Glycogen	0.41 $\pm$ 0.04	1.43 $\pm$ 0.08	0.001
Glucose	0.66 $\pm$ 0.05	3.13 $\pm$ 0.27	0.01
Glucose-6-phosphate	0.036 $\pm$ 0.002	0.141 $\pm$ 0.014	0.01
ATP	2.46 $\pm$ 0.05	1.39 $\pm$ 0.16	0.01
Lactate	0.95 $\pm$ 0.07	4.41 $\pm$ 0.39	0.01
$\sim P$	7.42 $\pm$ 0.15	13.18 $\pm$ 0.46	0.001

* n = 10.

† n = 4.

the other substrates were significantly higher in the cryptorchid testes. In addition, the concentration seemed to be slightly higher in the younger group of control animals.

Incubation experiments

Incubation of normal testes for 30 sec at the physiological temperature of the rat testis (33.6° C), and at a higher temperature (36.6° C) caused a rapid decrease in the glucose-6-phosphate concentration. After an incubation time of 1 min at 33.6° C, no significant decrease of the other substrate concentrations occurred but at 36.6° C, a marked lowering of ATP concentration was seen. Throughout the incubation experiments, the decrease of substrate concentrations differed only little in the testes incubated at the two different temperatures. The level of glycogen and glucose decreased to half in about 5 min at both temperatures and a half of both ATP and glucose-6-phosphate was utilized within about 1 min and 30 sec, respectively, at both temperatures (Text-figs. 1 and 2).

The experimentally cryptorchid testes behaved in a very different way during the incubation experiments, when compared with the control testes incubated at the same temperature of 36.6° C. The concentration of glycogen, which decreased only slowly in the normal testes, decreased from the high

TEXT-FIG. 1. Decrease of metabolite concentrations of normal and cryptorchid rat testis (○, normal testis at 36.6°C; ●, cryptorchid testis at 36.6°C) when incubated in the absence of oxygen. The animals were anaesthetized, the testes were excised and incubated in N₂-saturated mineral oil at temperatures indicated for the times given on the abscissa. The mean value for each time interval is derived from the number of tests shown in parentheses in Text-fig. 1(a). The vertical bars represent ± 1 S.E. (a) Glucose, (b) glycogen, (c) ATP, (d) glucose-6-phosphate.

TEXT-FIG. 2. Production of lactate by normal and cryptorchid rat testis when incubated in the absence of oxygen. The testes and the representation are the same as for Text-fig. 1.

initial value to one fourth of the value within 1 min. The levels of glucose-6-phosphate and ATP presented a very rapid decrease to half the original concentration within less than 30 sec. The initial level of glucose was much higher in the cryptorchid testes and the 50% value was attained only after 15 min instead of 5 min in the normal testes.

The rates of hexose utilization and lactate production were very similar in the normal testes at both incubation temperatures (Text-fig. 3). At 33.6° C,

TEXT-FIG. 3. Utilization of hexose (glycogen+glucose) and accumulation of lactate by normal and cryptorchid rat testis when incubated in the absence of oxygen. ○—○, Hexose, normal testis at 36.6° C; ○—○, $\frac{1}{2}$ lactate, normal testis at 36.6° C; ●—●, hexose, cryptorchid testis at 36.6° C; ●—●, $\frac{1}{2}$ lactate, cryptorchid testis at 36.6° C. The testes and the representation are the same as for Text-fig. 1.

there was a slight initial lag of lactate production at 1 min but later, the increase in lactate concentration accompanied the decrease in glucose and glycogen. At the incubation temperature of 36.6°C , the lag of lactate production lasted only 30 sec. In cryptorchid testes, production of lactate was seen to lag behind during the first 5 min but, later, hexose utilization and lactate production were observed in parallel in the testes.

An approximation of the testicular metabolic rate can be calculated in the terms of the rate of the use of high energy phosphate ($\sim\text{P}$) from the changes in ATP, glucose and glycogen. In the absence of O_2 , each mol of glucose converted to lactate produces 2 mol of $\sim\text{P}$. Each mol of glycogen converted to lactate produces 2.9 equivalents of $\sim\text{P}$, assuming a 10% branching in the glycogen molecule. ATP gives 2 equivalents of $\sim\text{P}$. In the presence of O_2 , for each mol of glucose oxidized to CO_2 , there is believed to be a yield of 38 equivalents of

TEXT-FIG. 4. Calculated use of high energy phosphate ($\sim\text{P}$) in normal and cryptorchid rat testis during anoxic incubation. The ordinate represents the sum of $\sim\text{P}$ derived from ATP, glucose and glycogen. ○, Normal testis at 36.6°C ; △, normal testis at 33.6°C ; ●, cryptorchid testis at 36.6°C .

$\sim\text{P}$ (i.e. an average P:O ratio of 3.2). At the incubation temperature of 33.6°C , a lower utilization of $\sim\text{P}$ by a normal rat testis was seen for the first 30 sec than at 36.6°C . During the first minute of incubation, however, the use of $\sim\text{P}$ was approximately the same in both groups, and at 5 min, the total amount used was near the exhaustion of the store. The cryptorchid testes were seen to utilize $\sim\text{P}$ at 36.6°C , at a rate which was twice as high as that of the normal testes. The amount utilized within 1 min was used as a basis for determination of the metabolic rate, because the testis is a relatively large organ and its initial oxygen content may influence the result considerably during the first few seconds. On the other hand, the value obtained for the cryptorchid testis is probably too low because of its very high initial consumption of $\sim\text{P}$, which nears the limits of exhaustion within 1 min (Text-fig. 4).

At the temperatures of 33.6°C and 36.6°C , the rates of use of $\sim\text{P}$ during

the first minute of anoxia were 173 and 160 mmol kg⁻¹ hr⁻¹, respectively, in the normal testis. On the basis of a P:O ratio of 3.2, this rate of ~P used is equivalent to an O₂ consumption of 27 and 25 mmol kg⁻¹ hr⁻¹, respectively. The cryptorchid testes consumed 318 mmol ~P kg⁻¹ hr⁻¹, which is equivalent to an oxygen consumption of 50 mmol kg⁻¹ hr⁻¹.

DISCUSSION

The seminiferous tubules and the interstitial tissue form two metabolically different tissue blocks, separated by the tubular supporting structures, which actively regulate the biochemical composition of extracellular fluid within the tubules (Setchell, Voglmayr & Waites, 1969). The net metabolic pattern and an experimentally induced change in this pattern may be the result of changes in the cellular composition or changes in the metabolic activity of the various cells themselves. The results of a biochemical study of the whole testis tissue have to be interpreted in the light of these relationships. In addition, pentobarbitone anaesthesia is known to depress the utilization of glucose by the testis (Setchell & Hinks, 1967) as well as by other parts of the body (Lowry *et al.*, 1964).

Pattern of recruitment of energy stores

The Sertoli cells contain the bulk of cytochemically demonstrable glycogen in the seminiferous tubules, both in normal (Fouquet & Guha, 1969) and in damaged tubules (Fabbrini, Re & Spera, 1969). The high increase of glycogen content may partially result from degeneration of those tubular cells which contain less glycogen.

The normal testis contained relatively little glucose when compared with the atrophic testis. The seminiferous tubules have a poor blood supply which inevitably results in a lower content of glucose than in the vascular interstitial tissue. There is no evidence of an increase in the permeability of the tubular wall in this condition (Kormano, 1968). Therefore, the relative increase in interstitial tissue and shrinkage of seminiferous tubules, seems to be responsible for the increased average glucose concentration of the whole organ. Another type of testicular damage, e.g. acute cadmium injury, does seem to increase the permeability of the tubular wall to glucose and consequently causes an increase in the average glucose concentration (Härkönen & Kormano, 1970). The ATP concentration of the normal testis was seen to be relatively high, approaching that of the nervous tissue (Lowry *et al.*, 1964). Considerable amounts of ATP have been demonstrated in spermatozoa and the compound is known to be related to sperm motility (Mann, 1964). This metabolite is probably largely formed during the late stages of spermatogenesis (Dawson, 1958). The decrease in the ATP content of cryptorchid testes may simply be a reflection of the disappearance of the more mature spermatogenic cells.

Lactate is known to be rapidly oxidized by the rat testis *in vitro* (Elliott, Greig & Benoy, 1937). Experiments *in vivo* on ram testis have also demonstrated that very little lactate is secreted into the venous blood of the testis (Waites & Setchell, 1964). Naturally cryptorchid ram testes, like the experimentally

cryptorchid rat testes in the present study, have a higher content of lactic acid than the scrotal organs (Blackshaw & Samisoni, 1967).

Metabolic differences between normal and cryptorchid testes

During incubation at two different temperatures, normal testes followed a similar pattern in the utilization of substrates, except for a slightly greater initial rate at the beginning of incubation at the higher temperature. The accumulation of lactate in anaerobic conditions followed closely the amount of hexose consumed. In cryptorchid testes, the glucose concentration decreased less rapidly than in the control testes and instead, a rapid mobilization of glycogen was seen. This suggests that there is an increased phosphorylase activity in the cryptorchid rat testis. As estimated histochemically, phosphorylase, both active and total, is confined to the seminiferous tubules and the rete testis (Guha & Wegmann, 1966). From the illustrations provided by these authors, it can be assumed that the localization of highest activity is in the Sertoli cells. Numerous other enzyme activities show a high increase histochemically in the tubular side of the rat testis (Kormano *et al.*, 1964), and it can be assumed that phosphorylase of Sertoli cells may be responsible for the very high activity in cryptorchid testes.

Gonadotrophins have been reported to increase the endogenous respiration of testis tissue and rat interstitial cells *in vitro* (Steinberger & Wagner, 1961; Hamberger & Steward, 1968), and to cause accumulation of cyclic AMP, which precedes the increase in hormone synthesis (Kuehl, Patanelli, Tarnoff & Humes, 1970). Since cyclic AMP, which seems to be a mediator of the hormonal activity, is also known to activate phosphorylase, one might also expect an increased phosphorylase activity in cryptorchid testes. Further evaluation of these factors will require more detailed studies, but it is interesting to note that in the hamster, testicular phosphorylase activity increases progressively during puberty (Fouquet & Guha, 1969). Fabbrini *et al.* (1969) have obtained histochemical evidence that in human testes with 'idiopathic' germinal cell aplasia, Sertoli cells may be congenitally lacking in glycogen and related enzymes but that in acquired germinal cell aplasia, a large amount of glycogen is present.

Glucose is the major energy substrate in both normal and cryptorchid testes, but there are different opinions on the effect of heat damage on glucose catabolism. The data of Hollinger & Davis (1968) and Free, Massie & VanDemark (1969) suggest that the overall oxidative catabolism of glucose is markedly reduced in cryptorchid rat testis and this is taken to reflect the absence of more mature germinal cells. In the conscious ram, Waites & Setchell (1964) observed an inconstant increase of glucose uptake by heated testes, but no change in the lactate concentration of the spermatic vein blood. In the aerobic incubation experiments of Hollinger & Davis (1968), cryptorchidism resulted in an increase in testicular protein labelling by radioactive glucose and impairment of glucose catabolism to perchloric-acid-soluble intermediates, among which lactate predominates.

The observations *in vitro* on the rat and ram testis agree with each other in that aerobically there is little change in the accumulation of lactate as a result of cryptorchidism, but anaerobic glycolysis is stimulated in the cryptorchid organ.

An elevation of 3° C in the incubation temperature will not immediately increase the internal temperature of the testis. This is certainly partially responsible for the small difference in the results between the two incubation temperatures. Waites & Setchell (1964) observed a 70% increase in the O₂ uptake of testes of conscious rams as a result of a 3° C rise in testicular temperature. Our numerical values for O₂ uptake (25 and 27 mmol kg⁻¹ hr⁻¹) are comparable with the observations made by Waites & Setchell, who observed a consumption of 13.2 to 16.2 mmol kg⁻¹ hr⁻¹ in a testis of a conscious ram at 34° C *in vivo*, and at a temperature of 37° C or more, they obtained a value of 22.0 mmol kg⁻¹ hr⁻¹. The O₂ consumption of the cryptorchid testis was seen to be about 100% higher than that of the normal testis at the same temperature. In the absence of added substrate, numerous authors have shown the elevation of the metabolic rate of the cryptorchid testis (Tepperman *et al.*, 1949). As a result of studies *in vitro* with cryptorchid rabbit testes, VanDemark & Ewing (1963) and Ewing & VanDemark (1963a, b) suggested that spermatogenic arrest, which results from exposure of the testis to elevated temperatures, may be caused by reduced levels of substrate in the tissue due to a fall in blood flow. The present results show that, with the exception of ATP, cryptorchid testes contained more substrates than normal testes. Our observations agree better with the suggestion of Waites & Setchell (1964) and Setchell, Waites & Thorburn (1966), that at least part of the testicular damage caused by heat *in vivo* may be due to hypoxia induced by an increase in O₂ consumption without a corresponding increase in the testicular blood flow. The rat testicular blood flow is little influenced by temperature elevation (Glover, 1966; Setchell *et al.*, 1969) and this results in severe hypoxia in the avascular seminiferous tubules.

The 100% difference between O₂ consumption by the normal and cryptorchid testis at the same temperature cannot be without significance. It may partly be due to hypophysial stimulation of the testicular cells which causes increased metabolic rate (Steinberger & Wagner, 1961). This possibility is supported by the observations of Hamberger & Steward (1968), who observed a 188% increase in the respiration of isolated Leydig cells as a result of stimulation with LH. Such a maximal stimulation in the present experiment seems improbable. Instead, when the O₂ consumptions of the normal rat testis (4.3 µl mg⁻¹ hr⁻¹ dry weight, Steinberger & Wagner, 1961) and the isolated interstitial-cell tissue, not subjected to hormone stimulation (7.28 to 17.99 µl mg⁻¹ hr⁻¹ dry weight, Hamberger & Steward, 1968) are compared, the higher basic metabolic rate of the interstitial tissue seems probable, though the opposite has also been suggested (Karjalainen & Niemi, 1969). Experiments are now in progress to demonstrate these possible differences.

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