AEROBIC METABOLISM OF UNIFORMLY LABELLED [¹⁴C]GLUCOSE IN TISSUE SLICES OF RAT TESTIS

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Summary. The catabolic fate of [U-¹⁴C]D-glucose has been studied under aerobic conditions of incubation in slices of mature scrotal, immature abdominal and experimentally-induced cryptorchid testes of the rat. In comparison with the mature scrotal testis, CO₂ production in the cryptorchid and the immature testis was found to be decreased by 45 and 55%, respectively. In contrast, protein labelling from radioactive glucose was found to be increased in the cryptorchid and the immature testis by a factor of 4.6 and 13.4, respectively. Successive ion-exchange chromatography of the perchloric acid-soluble fraction of the mature scrotal testis resulted in the elution of thirteen peaks of radio-activity from an anionic resin and five peaks of radio-activity from a cationic resin. The major catabolites occurring in slices of the mature scrotal testis were lactate, aspartate, glutamate, glutamine and α -ketoglutarate, accounting for 93% of the total perchloric acid-soluble radio-activity of glucose. Lactic acid accounted for 45.5, 36.9 and 15.9% in the mature scrotal, cryptorchid and immature testis, respectively. An additional unknown peak of radio-activity not found in samples of the mature scrotal testis was eluted from samples of both the immature abdominal and the experimentally-induced cryptorchid testis. It is suggested that of the cells of the seminiferous germinal epithelium, the maturing spermatids are characterized by the highest rate of glucose utilization into acid-soluble catabolites with a marked reduction in glucose catabolism occurring in the spermatogonia and primary spermatocytes.

INTRODUCTION

Previous results from this laboratory have indicated that the enzymatic systems involved in the incorporation of radio-active lysine into protein of testis slices are markedly more sensitive to the addition of exogenous glucose than those of a number of other tissues of the rat (Davis & Morris, 1963a; Davis & Firlit, 1965). The importance of glucose with regard to testicular metabolism has been

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further emphasized by the large reduction in oxygen uptake by the mature testis which results following either omission of glucose from the incubation medium (Schuler, 1944; Tepperman, Tepperman & Dick, 1949; Paul, Paul, Kopko, Bender & Everett, 1953; Serfaty & Boyer, 1956) or the induction of experimental hypoglycaemia in the animal (Ewing, Baird & VanDemark, 1966). In addition, the experimental induction of hypoglycaemia has been found to produce histological damage of the germinal epithelium of the rat testis with the atrophic changes occurring predominantly in the maturing spermatids (Mancine, Penhos, Izquierdo & Heinrich, 1960).

Reproductive function in the male has also been shown to be markedly influenced by the diabetic state with its associated presence of hyperglycaemia. The diabetic human male has been reported to have an increased incidence of impotence (Babbott, Rubin & Ginsburg, 1958; Rubin, 1958), low sperm counts (Schoffling, Federlin, Ditschuneit & Pfeiffer, 1963), a deficiency of spermatozoan motility (Klebanow & MacLeod, 1960) and atrophic changes in the seminiferous germinal epithelium of the testis (Schoffling *et al.*, 1963; Koch, 1910; Kraus, 1923). The experimental induction of hyperglycaemia in the male rat performed either by the administration of alloxan (Soulaire, Desclaux & Katz, 1948; Hunt & Bailey, 1961; Deb & Chatterjee, 1963) or by surgical pancreatectomy (Foglia, Borghelli, Chieri, Fernandez-Collazo, Spindler & Wesely, 1963) has been found to cause progressive testicular atrophy with resulting sterility of the animal. Moreover, a mutant strain of obese mice with hyperglycaemia also showed atrophic changes in the testis with a decrease in the number of spermatozoa (Ingalls, Dickie & Snell, 1950).

The possibility therefore exists that any alteration in the normal intracellular glucose level in the testis resulting from changes in blood sugar levels may cause an impairment of the spermatogenic process. Because of the marked dependency of the testis on glucose, it seemed of interest to attempt to establish normal metabolic patterns for glucose in various types of testicular tissue which might provide the basis for further investigations dealing with testicular malfunction. The present studies were designed to investigate the catabolic fate of glucose in slices of the immature testis, the mature testis and the cryptorchid testis of the rat, employing ion-exchange chromatography for the isolation of metabolites of uniformly-labelled glucose.

MATERIALS AND METHODS

Animals

The animals were male Sprague-Dawley rats obtained from the Abrams Small Stock Breeders, Chicago, Illinois, and fed Rockland rat diet freely. The surgical procedure for the experimental induction of unilateral cryptorchidism was performed in prepubertal rats which were 20 days of age, weighing 35 to 45 g and in which the testes had not yet begun their descent. The animals were killed and both scrotal and abdominal testes removed when the animals were 50 days of age and weighed 175 to 185 g. Immature testes located in the abdominal cavity were obtained from prepubertal rats which were 20 days of age when killed.

Experimental induction of cryptorchidism

Abdominal fixation of one of the undescended testes present in the 20-day-old rat was carried out under ether anaesthesia. A midline abdominal incision was performed and a fine 6-0 surgical silk suture was passed just underneath the tunica albuginea of the right testis and fixed to the dorsolateral abdominal wall, thereby preventing the normal descent of this testis. The left testis of the same animal was not sutured and was observed to descend into the scrotal sac when the animal reached approximately 35 days of age. Inasmuch as the suture was placed beyond the point where the testicular artery entered the parenchyma of the testis, no evidence of testicular ischaemia was observed during the time period studied.

Incubation of testicular slices with the tracer

After killing by decapitation, testes were rapidly removed at 4° C. Excision of the capsule was performed and slices of testes obtained with the aid of a Stadie-Riggs microtome. Incubation of the tissue slices was carried out in a Warburg apparatus as previously described (Davis, Firlit & Hollinger, 1963). The main chamber of the flask contained 300 mg wet weight of tissue in 3.0 ml of Krebs-Ringer bicarbonate buffer at pH 7.4. The side-arm contained 750,000 disintegrations/min of [$\text{U-}^{14}\text{C}$]D-glucose in a volume of 0.2 ml. The radio-active glucose was obtained from the Nuclear-Chicago Corporation and had a specific activity of 3.9 mc/mmmole. The final concentration of radio-active glucose in the incubation flask was 2.2×10^{-5} M. The gas phase was 95% O_2 and 5% CO_2 . At the end of a 1-hr incubation period at 37.5° C, the reaction was terminated by the addition of 0.5 ml of 5 N-perchloric acid.

Chromatography of the perchloric acid-soluble fraction

Following the addition of perchloric acid, the flask contents were centrifuged for 10 min at 600 g to separate the protein. The supernatant solutions from duplicate flasks were pooled and brought to pH 11.0 with KOH. After standing at 4° C for 2 hr, the insoluble potassium perchlorate was removed by centrifugation at 600 g. The samples were then added to a 12.5 $\times$ 0.9 cm column of Dowex 1-X8 ion-exchange resin in the acetate form, and elution carried out by an acetate gradient with a 200-ml mixing chamber initially filled with distilled water (Davis & Morris, 1963b). The reservoir contained 4 N-acetic acid during the collection of thirty 3-ml fractions, 8 N-acetic acid for the collection of thirty 5-ml fractions and a mixture of 8 N-acetic acid and 4 M-ammonium acetate during the collection of an additional thirty 5-ml fractions. The flow rate was adjusted to 1.5 ml/min by suitable air pressure. The material, which was not initially absorbed to the anionic Dowex 1 resin and which passed directly through this column, was brought to pH 2.5 with HCl and applied to a 17.0 $\times$ 0.9 cm column of Dowex 50W-X4 resin in the hydrogen form. Elution from this cationic resin was carried out by an HCl gradient with a 250-ml mixing chamber initially filled with distilled water. The reservoir contained 4 N-HCl during the collection of ninety 3-ml fractions. The flow rate was adjusted by air pressure to 1.0 ml/min. The material not adhering to either the anionic or

the cationic resin has been designated the neutral effluent and consists of the precursor, glucose (Busch, Fujiwara & Keer, 1960).

Assay of radio-activity

Following the collection of the effluent fractions, a 1-ml aliquot of each tube was transferred to counting vials and 15 ml of Bray's solution (Bray, 1960) added containing PPO (2,5-diphenyl-oxazole) and dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene). Samples were counted for 10 min in a Packard Tri-Carb liquid scintillation spectrometer. Corrections for quenching were determined both by the use of an internal [^{14}C]toluene standard as well as by the technique of automatic external standardization. The counting efficiency ranged from 64 to 67% at the beginning and the end of the acetate gradient, respectively, for effluent fractions from the Dowex 1 resin and from 42 to 67% at the beginning and the end of the HCl gradient, respectively, for effluent fractions from the Dowex 50 resin.

Determination of radio-activity in CO_2

At the end of the incubation period of the testicular slices with the tracer, the flasks were removed from their manometers and 0.5 ml of 1 M-hydroxide of hyamine-10X (Packard Instrument Company) placed in the centre well and 0.25 ml of 10 N-perchloric acid placed in the side-arm of each flask. The flasks were rejoined to the manometers and the perchloric acid tipped into the incubation medium in order to terminate the reaction and to evolve the $^{14}\text{CO}_2$ into the hyamine (Cuppy & Crevasse, 1963). The hyamine was then transferred to a counting vial containing 15 ml of 0.4% PPO and 0.01% dimethyl POPOP in toluene. Correction for quenching was determined by the use of an internal [^{14}C]toluene standard. The counting efficiency was 53%.

Determination of radio-activity in protein

After precipitation of the protein with perchloric acid and separation from the perchloric acid-soluble supernatant solution by centrifugation, the protein was washed with hot trichloroacetic acid, 95% ethanol, absolute ethanol and ether. The final precipitate was suspended in 2 ml of ether and placed in a tared counting vial, following which the ether was removed in a desiccator. The vial was reweighed and 1 ml of 1 M-hydroxide of hyamine-10X added. The vials were shaken at 60° C for 20 min (Hall, 1965), after which 15 ml of a scintillation medium consisting of 0.6% PPO and 0.03% dimethyl POPOP in toluene was added. Correction for quenching was determined by the use of an internal [^{14}C]toluene standard. The counting efficiency was 55%.

RESULTS

INCORPORATION OF RADIO-ACTIVITY INTO CO_2 AND PROTEIN

Table 1 presents the distribution of the non-perchloric acid-soluble radio-activity found in slices of the mature scrotal testis, the cryptorchid testis and the immature testis 1 hr after incubation with uniformly-labelled glucose. The

data for both $^{14}\text{CO}_2$ production and protein labelling from radio-active glucose are expressed in terms of disintegrations/min/100 mg wet weight of tissue. A 45% reduction in CO_2 production was found to occur in the experimentally-induced cryptorchid testis, while a 55% reduction in CO_2 production was observed in the immature testis. In marked contrast to these results, incorporation of radio-active glucose into protein of slices of both the cryptorchid testis and the immature testis was much greater than that found in the mature scrotal testis. Protein labelling from radio-active glucose was increased 4.6-fold in the cryptorchid testis and 13.4-fold in the immature testis.

TABLE 1

INCORPORATION OF RADIO-ACTIVITY INTO CO_2 AND PROTEIN IN TESTIS SLICES 1 HR AFTER INCUBATION WITH RADIO-ACTIVE GLUCOSE

	<i>Mature scrotal testis</i>	<i>Cryptorchid testis</i>	<i>Immature testis</i>
CO_2	46,400 (45,000 to 47,800)	25,800 (22,700 to 28,900)	20,925 (19,650 to 22,200)
Protein	1,126 (833 to 1,420)	5,155 (4,260 to 6,050)	15,100 (14,600 to 15,600)

The data are expressed in terms of disintegrations/min/100 mg tissue and represent the averages of duplicate experiments for each of the tissues studied. The ranges are presented in parentheses.

CHROMATOGRAPHIC PATTERNS OF PERCHLORIC ACID-SOLUBLE RADIO-ACTIVITY IN RAT TESTIS SLICES FOLLOWING AEROBIC INCUBATION WITH [$\text{U-}^{14}\text{C}$]GLUCOSE

Mature scrotal testis

Text-fig. 1 presents the chromatographic patterns of radio-activity from both Dowex 1 and Dowex 50 resins obtained from the perchloric acid-soluble fraction of the mature scrotal testis of the rat following incubation of slices with uniformly-labelled glucose. Thirteen peaks of radio-activity were eluted from the anionic Dowex 1 resin while elution of the cationic Dowex 50 resin gave an additional five peaks of radio-activity. Eight of these eighteen radio-active peaks have been identified by co-chromatography, paper electrophoresis and recrystallization to constant specific activity with authentic samples. These eight are alanine, peak 2; glutamic acid, peak 3; aspartic acid, peak 4; lactic acid, peak 5; succinic acid, peak 7; pyruvic acid, peak 11; α -ketoglutaric acid, peak 12; and glutamine, peak 15. The major catabolites of glucose in slices of the mature scrotal testis following aerobic incubation with the tracer therefore appear to be lactic acid, aspartic acid, glutamic acid, α -ketoglutaric acid and glutamine, with these five peaks accounting for 93% of the total perchloric acid-soluble metabolites of glucose.

Cryptorchid testis

Text-fig. 2 presents the chromatographic patterns of radio-activity from both Dowex 1 and Dowex 50 resins obtained from the perchloric acid-soluble

fraction of the cryptorchid testis of the rat following incubation of slices with uniformly-labelled glucose. Similar peaks of radio-activity as compared to the mature scrotal testis were eluted from the anionic Dowex 1 resin with two exceptions, namely the absence of succinic acid and peak 13. Elution of radio-activity from the cationic Dowex 50 resin when compared to the elution pattern obtained from the mature scrotal testis also revealed two qualitative differences which were the appearance of an additional peak of radio-activity eluted just prior to glutamine from samples of the cryptorchid testis and which has been designated as peak P-15 as well as the absence of peak 18 in the cryptorchid testis.

TEXT-FIG. 1. Chromatographic patterns of radio-activity of the perchloric acid-soluble fraction of the mature scrotal testis of the rat following incubation with radio-active glucose for 1 hr under aerobic conditions. Peak numbers 2, 3, 4, 5, 7, 11, 12 and 15 refer to alanine, glutamic acid, aspartic acid, lactic acid, succinic acid, pyruvic acid, α -ketoglutaric acid and glutamine, respectively. The remainder of the numbered peaks refer to unidentified catabolites. The data are the averages of three experiments. The positions of the peak tubes varied ± 1 fraction from the mean positions indicated in the chromatogram.

Immature testis

Text-fig. 3 presents the chromatographic patterns of radio-activity from both Dowex 1 and Dowex 50 resins obtained from the perchloric acid-soluble fraction of the immature testis of the rat following incubation of slices with uniformly-labelled glucose. The elution patterns of radio-activity obtained from samples of the immature testis for both anionic and cationic resins were qualitatively similar to those obtained from the cryptorchid testis. In a manner

TEXT-FIG. 2. Chromatographic patterns of radio-activity of the perchloric acid-soluble fraction of the cryptorchid testis of the rat following incubation with radio-active glucose for 1 hr under aerobic conditions. The data are the averages of five experiments. Also, see legend to Text-fig. 1.

TEXT-FIG. 3. Chromatographic patterns of radio-activity of the perchloric acid-soluble fraction of the immature testis of the rat following incubation with radio-active glucose for 1 hr under aerobic conditions. The data are the averages of four experiments. Also, see legend to Text-fig. 1.

closely related to the cryptorchid testis, the elution patterns of radio-activity in the immature testis demonstrated the same qualitative differences as compared to the mature scrotal testis, namely the absence of succinic acid, peak 13 and peak 18, as well as the appearance of an additional peak of radio-activity designated P-15.

Comparison of results obtained with the three types of rat testis

Table 2 presents a comparison of the specific activity of the catabolites of glucose in terms of disintegrations/min/100 mg wet weight of tissue which were found to occur in the perchloric acid-soluble fraction of the mature scrotal, the cryptorchid and the immature testis of the rat. In the mature scrotal testis, the

TABLE 2
DISTRIBUTION OF PERCHLORIC ACID-SOLUBLE RADIO-ACTIVITY IN TESTIS SLICES
1 HR AFTER INCUBATION WITH RADIO-ACTIVE GLUCOSE IN TERMS OF DISINTEG-
RATIONS/MIN/100 MG TISSUE

Chromatographic fraction	Mature scrotal testis	Cryptorchid testis	Immature testis
1 (unknown)	204 ± 101	678 ± 226	670 ± 112
2 (alanine)	420 ± 107	355 ± 21	936 ± 193
3 (glutamate)	14,280 ± 364	6,750 ± 806	5,650 ± 110
4 (aspartate)	21,100 ± 1,091	3,840 ± 471	2,010 ± 111
5 (lactate)	53,600 ± 7,600	28,750 ± 3,178	9,530 ± 937
6 (unknown)	2,140 ± 399	1,940 ± 99	1,040 ± 103
7 (succinate)	515 ± 73	0	0
8 (unknown)	564 ± 61	2,270 ± 258	3,780 ± 120
9 (unknown)	684 ± 40	163 ± 32	188 ± 63
10 (unknown)	1,400 ± 188	6,300 ± 553	7,400 ± 289
11 (pyruvate)	483 ± 67	2,700 ± 395	3,185 ± 325
12 (α-ketoglutarate)	7,350 ± 424	17,461 ± 1,030	6,645 ± 828
13 (unknown)	421 ± 310	0	0
14 (unknown)	90 ± 28	334 ± 116	401 ± 162
P-15 (unknown)	619 ± 61	3,860 ± 636	7,445 ± 320
15 (glutamine)	11,550 ± 1,640	10,279 ± 970	9,690 ± 910
16 (unknown)	286 ± 125	540 ± 35	610 ± 18
17 (unknown)	288 ± 84	277 ± 159	116 ± 39
18 (unknown)	176 ± 57	0	0
N.E. (glucose)	23,500 ± 4,100	67,800 ± 9,550	82,600 ± 5,190

Each value represents the mean ± standard error of three experiments for the mature scrotal testis, five experiments for the cryptorchid testis and four experiments for the immature testis.

specific activity of lactic acid, aspartic acid and glutamic acid was 53,600, 21,100 and 14,280 disintegrations/min/100 mg wet weight of tissue, respectively. A marked reduction in the incorporation of isotope into lactate, aspartate and glutamate was found to occur in both the cryptorchid and the immature testis, with the greatest reduction in lactate labelling from glucose occurring in the immature testis. In contrast, a marked increase in the amount of isotope in pyruvic acid was found to occur in both the cryptorchid and the immature testis. In comparison with the mature scrotal testis, isotope in succinic acid was found to be totally absent in the cryptorchid and the immature testis. Although appreciable radio-activity was found in glutamine in samples of the

mature scrotal, the cryptorchid and the immature testis, very little difference was found in the specific activity of labelled glutamine in these three tissues. On the other hand, the specific activity of glucose isolated from the cryptorchid testis was 2.9 times that found in the mature scrotal testis while the specific activity of glucose isolated from the immature testis was 3.5 times that found in the mature scrotal testis. These data indicate that there is a decreased utilization of glucose into perchloric acid-soluble catabolites in both the cryptorchid as well as the immature testis.

Table 3 presents a comparison of the catabolites of glucose occurring in the mature scrotal, cryptorchid and immature testis in terms of their percentages

TABLE 3
DISTRIBUTION OF PERCHLORIC ACID-SOLUBLE RADIO-ACTIVITY IN TESTIS SLICES
1 HR AFTER INCUBATION WITH RADIO-ACTIVE GLUCOSE IN TERMS OF PERCENTAGE
OF PCA-SOLUBLE METABOLITES OF GLUCOSE

Chromatographic fraction	Mature scrotal testis	Cryptorchid testis	Immature testis
1 (unknown)	0.20 $\pm$ 0.04	1.06 $\pm$ 0.47	1.11 $\pm$ 0.18
2 (alanine)	0.37 $\pm$ 0.16	0.42 $\pm$ 0.03	1.56 $\pm$ 0.30
3 (glutamate)	12.43 $\pm$ 1.06	8.19 $\pm$ 0.65	9.43 $\pm$ 0.23
4 (aspartate)	18.23 $\pm$ 1.29	4.95 $\pm$ 0.59	3.35 $\pm$ 0.17
5 (lactate)	45.54 $\pm$ 1.84	36.88 $\pm$ 1.68	15.89 $\pm$ 1.53
6 (unknown)	1.77 $\pm$ 0.35	2.27 $\pm$ 0.09	1.73 $\pm$ 0.18
7 (succinate)	0.43 $\pm$ 0.07	0	0
8 (unknown)	0.47 $\pm$ 0.11	2.89 $\pm$ 0.40	6.34 $\pm$ 0.21
9 (unknown)	0.59 $\pm$ 0.08	0.21 $\pm$ 0.04	0.31 $\pm$ 0.11
10 (unknown)	1.20 $\pm$ 0.11	7.95 $\pm$ 0.31	12.34 $\pm$ 0.46
11 (pyruvate)	0.45 $\pm$ 0.09	2.56 $\pm$ 0.44	6.38 $\pm$ 0.59
12 (α -ketoglutarate)	6.45 $\pm$ 0.83	21.63 $\pm$ 1.14	10.98 $\pm$ 1.38
13 (unknown)	0.35 $\pm$ 0.20	0	0
14 (unknown)	0.03 $\pm$ 0.01	0.43 $\pm$ 0.16	0.67 $\pm$ 0.28
P-15 (unknown)	0.53 $\pm$ 0.03	4.74 $\pm$ 0.66	12.43 $\pm$ 0.82
15 (glutamine)	9.85 $\pm$ 1.03	12.34 $\pm$ 0.78	16.16 $\pm$ 1.42
16 (unknown)	0.18 $\pm$ 0.02	0.67 $\pm$ 0.03	1.03 $\pm$ 0.04
17 (unknown)	0.26 $\pm$ 0.09	0.38 $\pm$ 0.24	0.20 $\pm$ 0.07
18 (unknown)	0.15 $\pm$ 0.04	0	0

Each value represents the mean $\pm$ standard error of three experiments for the mature scrotal testis, five experiments for the cryptorchid testis and four experiments for the immature testis.

of the perchloric acid-soluble radio-activity which was utilized by the tissue. In the mature scrotal testis, nearly one-half of the perchloric acid-soluble catabolites of glucose was accounted for by lactic acid, whereas in the cryptorchid and immature testes only 37 and 16%, respectively, of the perchloric acid-soluble catabolites of glucose occurred as lactic acid. In the cryptorchid testis, α -ketoglutaric acid was found to accumulate to the extent of accounting for 22% of the perchloric acid-soluble catabolites of glucose as compared to only 6% in the mature scrotal testis. Peak P-15, which was essentially absent in the mature scrotal testis appearing only as an ascending limb before the elution of glutamine, accounted for almost 5% of the perchloric acid-soluble intermediary metabolites of glucose in the cryptorchid testis and 12% of the

perchloric acid-soluble intermediary metabolites of glucose in the immature testis. Experiments are now in progress attempting to identify this peak which seems to occur predominantly in abdominal and immature testes.

DISCUSSION

The results of the present experiments indicate that the major catabolites of uniformly-labelled glucose occurring in slices of the mature scrotal testis of the rat incubated under aerobic conditions, are lactic acid, aspartic acid, glutamic acid, glutamine and α -ketoglutaric acid. These five catabolites were found to contain 93% of the total perchloric acid-soluble metabolites of glucose, with lactic acid alone accounting for almost one-half of the total perchloric acid-soluble metabolites of glucose. The fact that lactic acid was found to be the predominant catabolite of glucose occurring in the mature scrotal testis is in accordance with previous reports that the testis possesses a rapid rate of aerobic glycolysis (Warburg, 1927). The fact that glutamine was found to be a major catabolite of glucose in the mature scrotal testis may also be correlated with previous reports indicating a high activity of glutamine synthetase in the testis (Levintow, 1954). It is of interest to note that the remaining 7% of the total perchloric acid-soluble metabolites of glucose in the mature scrotal testis was distributed in thirteen peaks of radio-activity, with only alanine, succinic acid and pyruvic acid corresponding to known standards at the present time. The possibility therefore exists that some of the remaining ten presently unidentifiable peaks of radio-activity may represent new intermediates in the pathway of glucose degradation in the rat testis.

Employing the *in vitro* experimental conditions of the present report, the results indicate that the mature scrotal testis was capable of metabolizing 87.5% of the total radio-activity of uniformly-labelled glucose into CO_2 , protein and perchloric acid-soluble catabolites. In contrast, the cryptorchid testis and the immature testis had the capacity of metabolizing only 63.4 and 53.7%, respectively, of the total radio-activity into CO_2 , protein and perchloric acid-soluble catabolites. These data suggest that the overall oxidative catabolism of glucose is markedly reduced in both the cryptorchid and the immature testis of the rat. The appearance and activation of oxidative enzymes in the cells of the seminiferous germinal epithelium has been shown to correspond to the development of maturing spermatids (Ambadkar & George, 1964; Posalaky, 1965). Inasmuch as spermatids have been shown to be absent in both the immature and cryptorchid rat testis (Davis & Firlit, 1966), it would seem that a partial explanation for the decreased utilization of glucose observed in the immature and cryptorchid testis may involve the absence of these testicular cell types.

Lactic acid was found to account for 45.5% of the perchloric acid-soluble metabolites of glucose in the mature testis and only 15.9% of the perchloric acid-soluble metabolites of glucose in the immature testis. The reason for this difference in lactate accumulation by the testis with regard to age may be a

greater capacity of the immature testis to re-oxidize the lactate formed to pyruvic acid. Indeed, pyruvic acid was found to account for 6.4% of the perchloric acid-soluble metabolites of glucose in the immature testis and for only 0.4% in the mature testis.

The present experiments employing radio-active glucose also serve to confirm previous reports from this laboratory which have demonstrated an increase in protein labelling from radio-active lysine in both the cryptorchid and the immature testis of the rat as compared to a mature scrotal testis of the same animal (Davis, Morris & Hollinger, 1964, 1965). It was observed that a marked decrease of radio-activity in both aspartic acid and glutamic acid occurred in both the cryptorchid and the immature testis. One possible explanation for these results may be a more rapid incorporation of isotope from these two amino acids derived during the course of glucose catabolism into protein of the cryptorchid and immature testes.

A marked difference in the catabolic fate of glucose was observed between the mature scrotal testis and the experimentally-induced cryptorchid testis of the rat in that an additional peak of radio-activity, designated P-15, was eluted just before glutamine from samples of the cryptorchid testis, but not from samples of the mature scrotal testis. This additional peak of radio-activity was also found to occur in samples of the immature testis in an amount approximately three times the level seen in the cryptorchid testis. Experiments are now in progress which are designed to attempt to identify this unknown catabolite of glucose which is apparently unique to the cryptorchid testis and which also seems to disappear following the normal descent of the immature abdominal testis into the scrotal sac of the adult animal.

The data of the present experiments indicate that the induction of experimental cryptorchidism results in a marked increase in testicular protein labelling from radio-active glucose. In addition, the induction of cryptorchidism results in an impairment of glucose catabolism into acid-soluble intermediary metabolites by testicular tissue. The similarity in the metabolic fate of glucose observed in the cryptorchid testis and the immature testis further suggests that the maturing spermatids seem to be characterized by a low rate of protein biosynthesis that is greatly dependent on glucose utilization for its regulation. On the other hand, the spermatogonia and primary spermatocytes seem to be characterized by a much higher degree of protein synthesis that does not appear to be as dependent on glucose utilization. Any selective differences in the metabolism of those cells of the spermatogenic cycle responsible for cell proliferation and cell renewal as opposed to the spermatids which are undergoing morphogenic development may have importance in investigations on agents designed either to suppress or to enhance spermatogenesis. In addition, such selective metabolic differences may be of aid in the continuing search for potential side-effects of drugs affecting proliferating germinal cell systems with a possible resulting damage to the genome. The development of the presently described system for the study of glucose metabolites in testicular tissue would also seem to lend itself to further studies designed to explore such factors as hormones, drugs or potentially toxic chemicals found in the natural environment which may play a regulatory role in the catabolic fate

of glucose in the testis. Such experiments involving chemicals known to influence testicular morphology are currently being investigated in this laboratory.

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