NUCLEIC ACID LABELLING OF EXPERIMENTALLY-INDUCED CRYPTORCHID RAT TESTIS

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Summary. The *in vitro* incorporation of [2-¹⁴C]thymidine and [2-¹⁴C]-uridine into DNA and RNA respectively, has been studied in slices of immature, mature, cryptorchid, and contralateral-scrotal testes of the rat. The results demonstrate that DNA and RNA labelling are both enhanced in the cryptorchid testis following 30 days in the abdominal cavity. Of the four tissues studied, the immature testis possessed the highest *in vitro* rate of DNA and RNA labelling. In addition, uptake of [2-¹⁴C]uridine into RNA of the contralateral-scrotal testis of cryptorchid rats is elevated.

It is suggested that the enhanced nucleic acid labelling observed in the cryptorchid testis may be related to the predominance of those cells which normally synthesize nucleic acids during the cycle of the seminiferous epithelium, in association with compensatory gonadotrophin stimulation. Similarly, the increase in RNA labelling present in the contralateral scrotal testis may represent a compensatory 'feed-back' mechanism from the pituitary by virtue of decreased testosterone production in the cryptorchid testis. Increased gonadotrophin output in cases of cryptorchidism may be a complicating factor in the increased tendency of abdominal testes to undergo neoplastic change.

INTRODUCTION

Failure of the cryptorchid testis to achieve spermatogenesis appears to be a temperature-related phenomenon. However, the underlying mechanism involved in this effect remains incompletely defined. While numerous studies have been carried out dealing with nucleic acid synthesis in the mature scrotal testis, the possible involvement of altered nucleic acid synthesis in the abdominal testis has not been fully pursued. Changes in the concentration of various nucleic acid fractions in the experimentally-induced cryptorchid rat testis have been reported (McEneaney & Nelson, 1953). Recently, radio-autographic studies of DNA and RNA synthesis in experimentally-produced cryptorchid testes have been reported (Markewitz, Fingerhut & Veenema, 1966). DNA and RNA synthesis was detected in the germinal epithelium in the presence of

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degeneration of the seminiferous tubules and cessation of spermatogenesis. Neither of these studies has, however, attempted to quantitate the comparative nucleic acid biosynthetic capacity of cryptorchid testis tissue.

In addition to spermatogenic failure, several studies dealing with human testicular tumours indicate that testes retained in the ectopic abdominal position have a greater propensity for developing malignancies (Campbell, 1942; Sovhall, 1956). In fact, the undifferentiated cells present in human cryptorchid testes have been observed to resemble early carcinoma (Pace & Cabat, 1936). Previous results from this laboratory have demonstrated an increased *in vitro* protein labelling from radio-active lysine (Davis, Morris & Hollinger, 1964) and glucose (Hollinger & Davis, 1966) in the cryptorchid testis of the rat resembling the characteristic high rate of protein anabolism associated with tumour tissue.

The possibility, therefore, exists that any alteration in the biosynthesis of nucleic acids in the testis resulting from abdominal confinement may be related to impaired spermatogenesis and cryptorchid testis tumours. The present investigation has, therefore, been designed to investigate the effect of cryptorchidism on the *in vitro* incorporation of [2-¹⁴C]thymidine and [2-¹⁴C]uridine into DNA and RNA, respectively, of immature, mature, cryptorchid and contralateral scrotal rat testis slices.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Abrams Small Stock Breeders, Chicago, Illinois) 20, 30 and 60 days of age were used in the present study. Cryptorchid testes were prepared by suturing the right abdominal testis of 30-day prepubertal rats to the abdominal musculature as described previously (Davis, Morris & Hollinger, 1965). The abdominally-retained testis and the scrotal testis were removed 30 days after surgery. Control mature testes were obtained from 60-day-old rats in which no surgical manipulation had been performed.

Incubation

The procedure employed for incubation of testis slices was identical with that reported previously (Davis, Firlit & Hollinger, 1963). The gas phase was 95% O₂ and 5% CO₂ and the bath temperature was 32° C. Each flask contained 200 mg of tissue. The side-arm of the Warburg flasks contained 400,000 disintegrations/min of either [2-¹⁴C]thymidine or [2-¹⁴C]uridine (Nuclear-Chicago Corp.) in a volume of 0.2 ml.

Extraction of DNA and RNA

Isolation of the respective nucleic acid fractions was based on the technique described by Schneider (1957). Following homogenization at 4° C, 2.5 ml of 10 N-trichloroacetic acid (TCA) was added and the sample centrifuged at 600 *g* for 10 min at 4° C. The supernatant was discarded. Following an additional wash with 2.5 ml of 10 N-TCA the sediment was extracted with 5 ml of 95% ethanol. The RNA fraction was then isolated by adding 2 ml of 1 N-potas-

sium hydroxide, incubating overnight (20 hr) at 37° C, and centrifuging at 600 g for 10 min. The subsequent supernatant contained the dissolved RNA. DNA was recovered by heating the RNA-free sample with 2.6 ml of 5% TCA at 90° C for 15 min and centrifuging the soluble DNA off from the precipitated protein at 600 g for 10 min. The precipitate was then washed once with 2.5 ml of 5% TCA and the supernatants combined.

Assay of radio-activity

One-ml aliquots of each respective nucleic acid fraction were added to glass scintillation vials containing 15 ml of Bray's solution (Bray, 1960) and counted for 10 min in a Packard Tri-carb liquid scintillation spectrometer. Quench correction was determined by the addition of [^{14}C]toluene internal standard. Efficiency ranged from 67% to 72% with both the RNA and DNA fractions. Nearly 99% of all [$2\text{-}^{14}\text{C}$]thymidine was recovered in the DNA extract, while approximately 94% of the radio-activity of added [$2\text{-}^{14}\text{C}$]uridine was restricted to the RNA fraction.

Quantitation of DNA and RNA content

DNA and RNA were determined by colorimetric analysis of aliquots taken from the respective fractions. Standard DNA and RNA were obtained from the Sigma Chemical Company and were of calf thymus and yeast origin, respectively. The colorimetric determinations were based on the reactions of diphenylamine reagent with DNA producing a blue colour and read at 600 m μ , and orcinol reagent with RNA producing a green colour and read at 660 m μ in a Coleman Jr colorimeter (Schneider, 1957). Nearly 96% of all DNA recovered was found in the designated DNA fraction while recovery of RNA from the designated fraction amounted to 93% of the total RNA assayed.

RESULTS

The incorporation of [$2\text{-}^{14}\text{C}$]thymidine into testicular DNA following various periods of incubation at 32° C is presented in Text-fig. 1. A progressive increase in DNA labelling occurred from 0 to 60 min with an increased incorporation of the tracer still present up to 120 min. Text-fig. 2 presents the time course of the incorporation of [$2\text{-}^{14}\text{C}$]uridine into RNA of rat testis incubated at 32° C. The incorporation of [$2\text{-}^{14}\text{C}$]uridine into RNA was found to increase linearly up to 1 hr of incubation time, whereupon a gradual levelling off of incorporation of the tracer occurred. From these data, a 1-hr incubation period was chosen at a convenient time for studying *in vitro* nucleic acid labelling in slices of the rat testis.

The concentration of DNA present in the immature testis, the mature testis, the cryptorchid testis and the contralateral scrotal testis of the unilaterally-cryptorchid rat is presented in Text-fig. 3. The data obtained indicate that the immature testis contains the highest concentration of DNA and RNA of the four testicular tissues studied in terms of $\mu\text{g}/100$ mg wet weight tissue. The concentration of DNA in the cryptorchid testis and the contralateral scrotal testis of the same animal was not significantly altered from that of the control

TEXT-FIG. 1. Aerobic incorporation of [2-¹⁴C]thymidine into DNA of mature scrotal testis slices at various incubation periods. Each time point represents the average of two experiments.

mature testis. In a similar fashion, the cryptorchid testis and the control mature testis were found to contain essentially equal amounts of RNA. However, the concentration of RNA found in the contralateral scrotal testis of unilaterally-cryptorchid rats was observed to be significantly decreased by 34%.

TEXT-FIG. 2. Aerobic incorporation of [2-¹⁴C]uridine into RNA of mature scrotal testis slices at various incubation periods. Each time point represents the average of two experiments.

Text-fig. 4 presents the incorporation of $[2-^{14}\text{C}]$ thymidine into DNA of slices of the immature testis, the mature testis, the cryptorchid testis and the contralateral scrotal testis of the unilaterally-cryptorchid rat expressed as disintegrations/min/mg DNA. The specific activity of DNA as compared to the

TEXT-FIG. 3. Concentration of DNA and RNA in the 20-day immature testis, the 60-day mature testis, the 30-day cryptorchid testis and the contralateral scrotal testis of a unilaterally-cryptorchid rat. Each bar represents the average of three experiments $\pm$ S.E. with the exception of the cryptorchid testis which represents the average of five experiments.

control mature testis was found to be increased by a factor of 3.1 in the immature testis and by a factor of 1.5 in the cryptorchid testis. However, incorporation of $[2-^{14}\text{C}]$ thymidine into DNA of the contralateral scrotal testis of the unilaterally-cryptorchid rat was found to be decreased by 31% when compared with the

TEXT-FIG. 4. Aerobic incorporation of $[2-^{14}\text{C}]$ thymidine and $[2-^{14}\text{C}]$ uridine into DNA and RNA, respectively, of slices of the 20-day immature testis, the 60-day mature testis, the 30-day cryptorchid testis and the contralateral scrotal testis of a unilaterally-cryptorchid rat. Each bar represents the average of three experiments $\pm$ S.E. with the exception of the cryptorchid testis which represents the average of five experiments.

control mature testis of an intact rat. The Text-figure 4 also presents the incorporation of [2-¹⁴C]uridine into RNA of slices of the immature testis, the mature testis, the cryptorchid testis and the contralateral scrotal testis of the unilaterally-cryptorchid rat expressed as disintegrations/min/mg RNA. RNA labelling was found to be markedly elevated in the immature, the cryptorchid and the contralateral scrotal testis of the unilaterally-cryptorchid rat as compared with the control mature testis. The specific activity of RNA as compared with the control mature testis was increased by a factor of 2.8 in the immature testis, by a factor of 2.3 in the cryptorchid testis and by a factor of 2.0 in the contralateral-scrotal testis of the unilaterally-cryptorchid rat.

DISCUSSION

The results of the present study indicate that the artificially-induced rat cryptorchid testis is capable of synthesizing nucleic acids as measured by the *in vitro* incorporation of [2-¹⁴C]thymidine and [2-¹⁴C]uridine into DNA and RNA, respectively. Furthermore, both DNA and RNA labelling are increased in the undescended testis, as compared to the mature scrotal testis. The incorporation of radio-active thymidine into DNA of the cryptorchid testis was enhanced 48% while uptake of radio-active uridine into RNA was increased by a factor of 2.4. In view of the fact that the concentrations of DNA and RNA in the cryptorchid testis were not significantly different from those of the mature scrotal testis, the increased uptake of labelled thymidine and uridine may reflect a more rapid turnover of nucleic acids in this tissue.

The data of the present experiments also indicate that the incorporation of [2-¹⁴C]thymidine and [2-¹⁴C]uridine into DNA and RNA, respectively, of the immature testis of the rat is markedly enhanced. Spermatogenesis in the 20-day immature testis has not yet proceeded beyond the primary spermatocyte stage (Davis *et al.*, 1964). In a similar manner, the interference of normal testicular descent in the 30-day-old rat results in failure of spermatogenesis to proceed beyond the primary spermatocyte stage following 30 days in the abdominal cavity (Davis & Firlit, 1966). DNA and RNA synthesis are known to occur at discrete periods during the cycle of the seminiferous epithelium. DNA anabolism is greatest in preleptotene spermatocytes (Monesi, 1962), while RNA synthesis is most marked during middle (Monesi, 1964) and late pachytene spermatocytes (Utakaji, 1966). It would therefore appear possible that a partial explanation for the increased *in vitro* labelling of both DNA and RNA in the cryptorchid and immature testis of the rat is related to the predominance of the early generation cells of the seminiferous epithelium which are concerned with the normal synthesis of nucleic acids.

An additional factor possibly involved in the regulation of testicular nucleic acid synthesis concerns the gonadotrophins. In addition to the increase in DNA and RNA labelling observed in the cryptorchid testis, RNA labelling was increased in the contralateral scrotal testis, while the concentration of RNA in this tissue was decreased. These results indicate an alteration in RNA metabolism in the scrotal testis of cryptorchid rats. Compensatory morphological changes have been described in the contralateral scrotal testis of cryptorchid

rats (Clegg, 1965). These changes include hypertrophy of germ cells in the absence of hyperplasia, and hypertrophy accompanied by hyperplasia of Leydig cells. The mean diameter of pachytene spermatocytes at Stage VII was found to be significantly greater in the scrotal testis from unilaterally-cryptorchid rats. It was suggested that these changes are related to increased release of both follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) from the pituitary. Hall & Eik-Nes (1962) have demonstrated that ICSH is capable of stimulating the incorporation of $[1-^{14}\text{C}]$ valine and $[1-^{14}\text{C}]$ -tryptophane into protein of rabbit testis slices whether administered *in vivo* or *in vitro*. It would appear possible that following arrest of spermatogenesis and inhibition of testosterone synthesis (Eik-Nes, 1966; Llaurodo & Dominquez, 1963) in the cryptorchid testis, a compensatory release of gonadotrophins might induce an increase in RNA synthesis in the cryptorchid testis which is also reflected in the contralateral scrotal testis.

The most likely substance responsible for the increased RNA synthesis would appear to be FSH. It has recently been postulated that Leydig cells may regulate the release of FSH from the pituitary (Steinberger & Duckett, 1966). In view of the evidence of recent investigators on the effect of cryptorchidism on testosterone production by the Leydig cells, it would seem that a decrease of testosterone production approximating to 60 to 80% of normal levels is brought about. Therefore, a corresponding compensatory release of FSH might be expected. The contemporary view of the biochemical mechanism of action of hormones suggests a possible involvement in the regulation of RNA synthesis.

Reflex release of gonadotrophins in cases of cryptorchidism may also be related to the increased incidence of neoplasia in abdominally-retained testes. Zimel, Macrineanu & Hillebrand (1962) have observed that hypophysectomy inhibits the rate of tumour transplant growth within the testis and the extent to which metastases occur. Hypophysectomy was also observed to abolish the enhanced tendency of tumours to develop and grow in experimentally-induced cryptorchid testes. It appears possible that compensatory gonadotrophin release following the induction of cryptorchidism may be a complicating factor which potentiates neoplastic change in the ectopic testis by stimulating RNA synthesis and subsequent cytoplasmic growth. Future studies dealing with the effect of gonadotrophins on testicular nucleic acid synthesis are at present being initiated.

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