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SHORT COMMUNICATIONS

Effect of nitrofurazone on the incorporation of L-lysine-U-^{14}C into protein of rat testis*

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THE nitrofuran group of chemotherapeutic agents has long been known to exert specific histological changes in the testicular germinal epithelium of the rat,[1] mouse,[2] and dog.[3] These changes involve the arrest of spermatogenesis at the primary spermatocyte stage.[4] The histological architecture of the nitrofuran-treated testis of the rat therefore closely resembles that of the cryptorchid rat testis in being characterized by a virtual absence of all the spermatids and mature spermatozoa. Recent data from this laboratory have indicated that the induction of experimental cryptorchidism in the rat results in a marked increase in the incorporation of L-lysine-U-^{14}C into protein of the remaining cells of the seminiferous germinal epithelium.[5, 6]

It appears that the loss of spermatids and spermatozoa from a nitrofuran-treated testis offers a unique opportunity to make a selective study of protein labeling of the remaining cells of the seminiferous epithelium as they exist in the natural environmental temperature of the scrotal sac rather than as they exist after exposure to the higher environmental temperature of the abdominal cavity as is necessarily the case in experimental cryptorchidism. The present studies were therefore designed to investigate the incorporation of radioactive lysine into protein of the remaining cells of the testis after the oral administration of nitrofurazone to adult rats.

MATERIALS AND METHODS

The animals used in these experiments were male Sprague-Dawley rats (185–220g) approximately 60 days old, obtained from the Abrams Small Stock Breeders, Chicago, Ill. Nitrofurazone (generously supplied by Eaton Laboratories, Norwich, N.Y.) was added to an *ad libitum* diet for a period of 30 days at a concentration of 0·1% in Rockland powdered rat chow. In addition, nitrofurazone was also suspended in 0·3% carboxymethylcellulose and 1 mg/kg given daily by oral gavage for 20 days. Control rats received both Rockland powdered rat chow and carboxymethylcellulose without nitrofurazone, respectively.

At the end of the desired experimental period, the animals were sacrificed by decapitation and slices of testes obtained with the aid of a Stadie-Riggs microtome at 4°. Incubation of the testis slices with radioactive lysine was carried out in a Warburg apparatus as previously described.[7] The main chamber of the Warburg flask contained 200 mg tissue in 3·0 ml Krebs-Ringer bicarbonate buffer at pH 7·4. The side arm contained $2{\cdot}5 \times 10^5$ counts/min of L-lysine-U-^{14}C (specific activity 5·8 mc/mmole) in a volume of 0·2 ml. The final concentration of L-lysine-U-^{14}C in the incubation flask was $1{\cdot}8 \times 10^{-5}$ M. The gas phase was 95% O_2 and 5% CO_2. An incubation temperature of 34° was employed.[8] At the end of a 1-hr incubation period, the reaction was terminated by the addition of 0·3 ml of 5 N perchloric acid. The proteins were isolated, plated, and assayed for radioactivity, with a correction for self-absorption. Evidence that the uptake of radioactive lysine into protein is due to incorporation of the amino acid in peptide linkage and not due to a nonenzymatic N-terminal acylation was obtained by reacting the protein with 1-fluoro-2,4-dinitrobenzene. After hydrolysis and ether extraction of the dinitrophenyl–protein, aliquots of the aqueous phase were chromatographed on paper in two different solvent systems; the R_f for the radioactive dinitrophenyl amino acid was identical with that of N-dinitrophenyl-ε-lysine.

RESULTS

The changes in the weight of testes obtained from adult rats receiving 0·1% nitrofurazone in the diet for 30 days are illustrated in Fig. 1. A progressive decrease in the weight of testes from

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nitrofurazone-fed animals occurred up to 30 days, after which testicular weights reached constant values. Testes from control rats fed the same diet but without added nitrofurazone showed a normal small increase in weight from 60 to 90 days of age. At the end of the experimental period, the weights of the nitrofurazone-treated testes averaged one third that of control values. No significant difference in total body weight was observed between the nitrofurazone-fed animals and the control animals throughout the experimental period.

Figure 1 also presents the effect of nitrofurazone at 0·1% of the diet on the incorporation of L-lysine-U-^{14}C into protein of testis slices at various times during the feeding period. The uptake of labeled lysine into protein per milligram dry weight of protein of testis slices obtained from rats fed nitrofurazone progressively increased up to 30 days, at which time the uptake of isotope into protein was four times that observed for testicular slices from control rats. The incorporation of labeled lysine into protein of slices of testes obtained from control rats remained constant throughout the 30-day experimental period.

TABLE 1. EFFECT OF ORAL ADMINISTRATION OF NITROFURAZONE ON THE INCORPORATION OF L-LYSINE-U-^{14}C INTO PROTEIN OF ADULT RAT TESTIS SLICES

	Body weight (g)	Testicular weight (mg)	Specific activity of protein (counts/min/mg protein)
Control	312	1690	438
Nitrofurazone	325	801	1656

Nitrofurazone was suspended in 0·3% carboxymethylcellulose and administered (1 mg/kg) to 60-day-old rats by oral gavage daily for 20 days. For experimental details see legend for Fig. 1. Each value represents the average of two experiments.

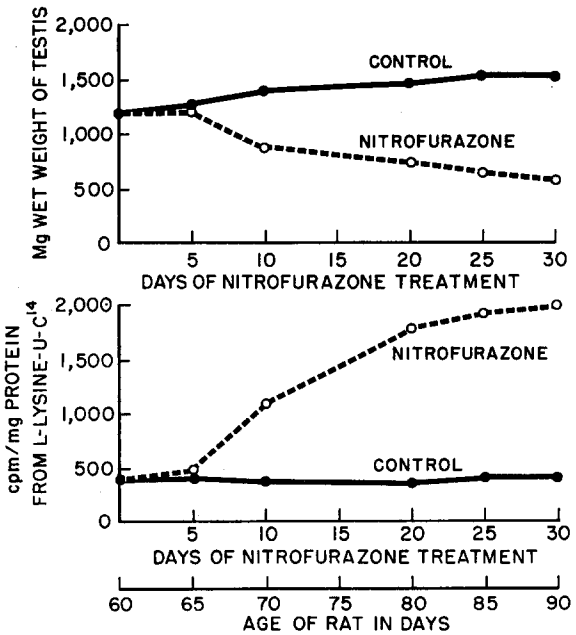

FIG. 1. Effect of 0·1% nitrofurazone in diet for a period of 30 days on the incorporation of L-lysine-U-^{14}C into protein of adult rat testis slices. Flask contents: 250,000 counts/min of L-lysine-U-^{14}C; Krebs-Ringer bicarbonate buffer, pH 7·4; to a total volume of 3·2 ml. Gas phase 95% O_2 and 5% CO_2; incubation 1 hr; incubation temperature 34°. Each point on the curves represents the average of four experiments.

Table 1 indicates the results of similar experiments carried out after the daily oral administration of 1 mg nitrofurazone/kg to adult rats for 20 days. Testicular weights of rats given nitrofurazone *per os* for 20 days were decreased by 50 per cent as compared to controls. The incorporation of radioactive lysine into testicular protein of rats given nitrofurazone orally increased from control values averaging 438 counts/min/mg protein to a specific activity that averaged 1656 counts/min/mg protein.

DISCUSSION

Nitrofurans have previously been shown not only to cause the loss of spermatids and mature spermatozoa from the seminiferous tubules of the rat testis but to impair both glucose utilization as well as pyruvate oxidation in the rat testis.[9–11] These studies have suggested a marked metabolic dependency of the spermatids and mature spermatozoa of the testis on aerobic glucose oxidation. In addition, radioautographic data from this laboratory on the effect of glucose on the uptake of tritiated lysine into cells of the seminiferous epithelium have demonstrated that, of the various spermatogenic cell types found in the rat testis, the addition of exogenous glucose caused the greatest degree of stimulation of protein labeling in the spermatids.[12]

The data of the present experiments indicate that the loss of spermatids and mature spermatozoa from the seminiferous tubules of the rat testis resulting from the oral administration of nitrofurazone is accompanied by a marked increase in testicular protein labeling from radioactive lysine. It appears that the remaining cells of the seminiferous germinal epithelium found at the lower physiological environmental temperature of the scrotal sac after the administration of nitrofurazone are responsible for the observed increase in testicular protein labeling and that these cells are the spermatogonia and primary spermatocytes that are also responsible for cell renewal in the spermatogenic cycle. It is therefore possible to state at the present time that while the spermatids and mature spermatozoa seem to be characterized by a greater dependency on glucose utilization for their morphological integrity, the spermatogonia and primary spermatocytes are characterized by a much greater degree of protein biosynthesis, which appears to increase markedly owing to the action of nitrofurazone on these testicular cell types. Any difference in the metabolism of those cells of the spermatogenic cycle responsible for cell proliferation and cell renewal as compared to the more mature spermatids and spermatozoa may have importance in investigations on spermicidal agents as well as for potential side effects of drugs affecting proliferating germinal cell systems with a possible resulting damage to genetic material.

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REFERENCES

1. J. T. PRIOR and J. H. FERGUSON, *Cancer* **3**, 1062 (1950).
2. J. A. NISSIM, *Lancet* **i**, 304 (1957).
3. G. S. ROGERS, G. B. BELLOFF, M. F. PAUL, J. A. YURCHENCO and G. GEVER, *Antibiotics and Chemother.* **6**, 231 (1956).
4. W. O. NELSON and E. STEINBERGER, *Anat. Rec.* **112**, 367 (1952).
5. J. R. DAVIS, R. N. MORRIS and M. A. HOLLINGER, *Am. J. Physiol.* **207**, 50 (1964).
6. J. R. DAVIS, R. N. MORRIS and M. A. HOLLINGER, *J. Reprod. Fert.* **10**, 149 (1965).
7. J. R. DAVIS, C. F. FIRLIT and M. A. HOLLINGER, *Am. J. Physiol.* **204**, 696 (1963).
8. J. R. DAVIS and R. N. MORRIS, *Am. J. Physiol.* **205**, 833 (1963).
9. H. E. PAUL, M. F. PAUL, F. KOPKO, R. C. BENDER and G. EVERETT, *Endocrinology* **53**, 585 (1953).
10. M. F. PAUL, H. E. PAUL, F. KOPKO, M. J. BRYSON and C. HARRINGTON, *J. biol. Chem.* **206**, 491 (1954).
11. R. M. FEATHERSTONE, W. O. NELSON, F. WELDEN, E. MARBERGER, A. BOCCABELLA and R. BOCCABELLA, *Endocrinology* **56**, 727 (1955).
12. J. R. DAVIS and C. F. FIRLIT, *Am. J. Physiol.* **209**, 425 (1965).