EFFECT OF NITROFURAZONE ON THE AEROBIC METABOLISM OF UNIFORMLY LABELLED [^{14}C]GLUCOSE IN TISSUE SLICES OF RAT TESTES

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Previous data from this laboratory have indicated that the change in protein labelling of slices of experimentally-induced cryptorchid rat testes and testes from rats fed nitrofurazone is similar in that the incorporation of L-lysine- $\text{U-}^{14}\text{C}$ progressively increases during both experimental periods (Davis, Morris & Hollinger, 1964; Hollinger & Davis, 1966). More recent data from this laboratory have indicated that while there is a reduced capacity to catabolize D-glucose- $\text{U-}^{14}\text{C}$ into acid-soluble intermediates, protein labelling from radio-active glucose is markedly elevated in the cryptorchid testis (Hollinger & Davis, 1968).

The atrophic changes in the testis of the nitrofurazone-treated rat closely resemble those in the experimentally-induced cryptorchid rat testis in that both testes show an absence of spermatids and mature spermatozoa. It seemed of interest, therefore, to study the glucose metabolism of the nitrofurazone-treated testis which appeared morphologically cryptorchid at the naturally-occurring lower temperature of the scrotal sac.

The animals used in these experiments were 60-day-old male Sprague-Dawley rats, weighing approximately 185 to 200 g. Nitrofurazone was added to an unrestricted diet of Rockland powdered rat chow for a period of 30 days at a concentration of 0.1%. Control rats received the same diet without added nitrofurazone. At the end of the 30-day experimental feeding period, aerobic incubation of testicular slices with 750,000 disintegrations/min of D-glucose- $\text{U-}^{14}\text{C}$ for 1 hr at 37.5°C was carried out in a Warburg apparatus as previously described (Davis, Firlit & Hollinger, 1963). Radio-activity in CO_2 and protein, as well as ion-exchange chromatography of the perchloric acid-soluble supernatant solution was performed as previously described (Hollinger & Davis, 1968).

The chromatographic patterns of radio-activity obtained from the normal adult testis of a 90-day-old rat are shown in the two top graphs of Text-fig. 1. Thirteen radio-active peaks were eluted from the Dowex-1 column while elution of the Dowex-50 column gave an additional five peaks of radio-activity. The following peaks have been identified by co-chromatography, paper

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TEXT-FIG. 1. Chromatographic patterns of the radio-activity of the perchloric acid-soluble fraction of slices of the normal adult rat testis (upper graphs) and the nitrofurazone-treated rat testis (lower graphs) following incubation with D-glucose-U- ^{14}C 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 four experiments. The positions of the peak tubes varied ± 1 fraction from the mean positions indicated in the chromatogram.

electrophoresis and recrystallization to constant specific activity with authentic samples: peak 2, alanine; peak 3, glutamic acid; peak 4, aspartic acid; peak 5, lactic acid; peak 7, succinic acid; peak 11, pyruvic acid; peak 12, α -keto-glutaric acid and peak 15, glutamine.

The two bottom graphs of Text-fig. 1 present the chromatographic patterns of radio-activity obtained from the perchloric acid-soluble fraction of testes from 90-day-old rats which had been previously fed 0.1% nitrofurazone in the diet for 30 days. Elution of the Dowex-1 sample revealed eight radio-active peaks while elution of the Dowex-50 column demonstrated the presence of five radio-active peaks including the appearance of an additional labelled

TABLE 1
DISTRIBUTION OF PERCHLORIC ACID-SOLUBLE RADIO-ACTIVITY
IN TESTIS SLICES 1 HR AFTER INCUBATION WITH D-GLUCOSE-U-
 ^{14}C IN TERMS OF DISINTEGRATIONS/MIN/100 MG TISSUE

Chromatographic fraction	Control adult rat testis	Nitrofurazone-treated adult rat testis
1 (unknown)	222 $\pm$ 27	210 $\pm$ 7
2 (alanine)	318 $\pm$ 179	0
3 (glutamate)	12700 $\pm$ 237	3240 $\pm$ 273
4 (aspartate)	23400 $\pm$ 1481	3072 $\pm$ 376
5 (lactate)	51900 $\pm$ 1365	31800 $\pm$ 638
6 (unknown)	4400 $\pm$ 757	0
7 (succinate)	498 $\pm$ 48	0
8 (unknown)	426 $\pm$ 32	1960 $\pm$ 188
9 (unknown)	428 $\pm$ 24	0
10 (unknown)	1280 $\pm$ 84	4560 $\pm$ 352
11 (pyruvate)	766 $\pm$ 273	3180 $\pm$ 244
12 (α -ketoglutarate)	4450 $\pm$ 310	2690 $\pm$ 203
13 (unknown)	145 $\pm$ 44	0
14 (unknown)	80 $\pm$ 21	295 $\pm$ 29
P-15 (unknown)	865 $\pm$ 347	4800 $\pm$ 722
15 (glutamine)	15200 $\pm$ 449	10690 $\pm$ 1468
16 (unknown)	57 $\pm$ 25	465 $\pm$ 49
17 (unknown)	148 $\pm$ 66	227 $\pm$ 23
18 (unknown)	83 $\pm$ 43	0
N.E.† (glucose)	23100 $\pm$ 1580	133000 $\pm$ 8255

* Each value represents the mean $\pm$ standard error of three experiments for the control adult rat testis and four experiments for the nitrofurazone-treated adult rat testis.

† N.E. = neutral effluent

metabolite which has been designated P-15, the elution of which occurs just before that of glutamine. The following radio-active peaks were found to be absent from the perchloric acid-soluble fraction of the nitrofurazone-treated testis: alanine; succinate and unknown peaks 6, 9, 13 and 18.

The distribution of the labelled catabolites of glucose occurring in the perchloric acid-soluble fraction obtained from slices of the control adult testis and the nitrofurazone-treated testis is presented in Table 1. A comparison of the catabolites indicated reduced amounts of aspartate, glutamate, α -ketoglutarate, lactate and glutamine and complete absence of alanine and succinate in the nitrofurazone-treated testis. By contrast, the amount of isotope occurring in pyruvate was found to be increased 4.2-fold in the nitrofurazone-treated

testis. The neutral effluent, consisting of the precursor, D-glucose-U- ^{14}C , contained approximately six times more radio-activity in the nitrofurazone-treated testis than did the control, indicating a markedly decreased utilization of glucose in testes from animals fed nitrofurazone.

Table 2 presents the distribution of the non-perchloric acid-soluble radio-activity found in slices of the control adult testis and the nitrofurazone-treated testis 1 hr after incubation with uniformly-labelled glucose. A 78% reduction in radio-active carbon dioxide production was found to occur in testes from rats which received nitrofurazone. In contrast to these results, the incorporation of D-glucose-U- ^{14}C into protein in slices of nitrofurazone-treated testes was 3.7 times as great as that found in the control adult testis.

The data of the present investigations indicate that the chronic administration of 0.1% nitrofurazone in the diet results in a decreased metabolism of D-glucose-U- ^{14}C by rat testicular tissue. This observation is in accord with

TABLE 2
DISTRIBUTION OF NON-PERCHLORIC ACID-SOLUBLE RADIO-
ACTIVITY IN TESTIS SLICES 1 HOUR AFTER INCUBATION
WITH D-GLUCOSE-U- ^{14}C

	<i>Control adult rat testis</i>	<i>Nitrofurazone-treated adult rat testis</i>
CO_2	47,375 (46,400 to 50,200)	10,498 (8260 to 11890)
Protein	1488 (1126 to 1850)	5433 (4980 to 6340)

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

previously reported biochemical and morphological effects caused by nitrofurans (Paul, Paul & Kopko, 1952; Paul, Paul, Kopko, Bender & Everett, 1953). The resulting inhibition of testicular oxidative metabolism by nitrofurazone with a subsequent depletion of the glucose-dependent spermatids would therefore be expected to cause the observed decreased utilization of radio-active glucose in the nitrofurazone-treated testis. The relative independence of the spermatogonia for glucose metabolism (Davis & Firlit, 1965) may partially explain the refractiveness of these undifferentiated germ cells to morphological damage in conditions of hypoglycaemia (Mancini *et al.*, 1960).

The increased *in vitro* testicular protein labelling from uniformly-labelled glucose following treatment of the animal with nitrofurazone could be due to a direct stimulatory effect of the drug on the protein-synthesizing systems present in the testis or might reflect the drug-induced depletion of spermatids and spermatozoa which synthesize very little protein, with a consequent unmasking of the more rapid protein-synthesizing systems present in the spermatogonia and primary spermatocytes. The incorporation of tritiated lysine into successive cells of the cycle of the seminiferous epithelium in the rat has been reported by Davis & Firlit (1965) to be highest in spermatogonia and, particularly, the

early stages of the primary spermatocytes. The data obtained employing the nitrofurazone-treated testis of the rat which is devoid of spermatids in much the same manner as in the cryptorchid rat testis further suggest that different cells of the spermatogenic cycle have different metabolic capacities. The spermatogonia and primary spermatocytes, by contrast with the maturing spermatids, seem to be characterized by a much greater degree of protein synthesis that does not appear to be very dependent on glucose utilization. It appears that the effects both of an increased abdominal environmental temperature and nitrofurazone administration result in similar changes of glucose catabolism and protein labelling in the rat testis. In addition, the present studies suggest that the administration of nitrofurazone may be associated with subtle biochemical side-effects which cannot be noticed by employing ordinary histological testicular biopsy techniques.

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