METABOLIC EFFECTS OF $\alpha$-TOCOPHERYL ACETATE
IV. DEOXYRIBONUCLEIC ACID OF THE RAT LIVER CELL,
FOLLOWING DAILY DIETARY SUPPLEMENTS OF
$\alpha$-TOCOPHERYL ACETATE

DORIS E. GRAY, JAMES CHISHOLM AND C. H. LEE PENG

Department of Biochemistry, University of Hong Kong, Hong Kong

(Received July 18, 1960)

It is well-established that amount of deoxyribonucleic acid (DNA) per somatic cell for a given species remains constant, irrespective of the nutritional status of the animal (1). Therefore, any variation in the mean amount of DNA per organ must be attributed to variations in either the mass or the number of cells of the tissue concerned. In the course of an investigation into the activity of certain enzymes of rat liver following a period of daily administration of $\alpha$-tocopheryl acetate ($\alpha$TAc) it was observed that the DNA content per unit weight of liver was significantly increased. This paper deals with the findings in respect of DNA, and the calculation from these data, of liver cell number and cell mass. Paper V, which follows the present paper, is concerned with the enzyme activities, and other metabolic determinations, from the same experimental material.

EXPERIMENTAL

Male weaning rats were divided into three groups and maintained for periods of four to six weeks on a vitamin E-free, low fat diet of the following composition, by weight: carbohydrate 67%, fat 3%, and protein 27%. Two of the groups were given daily supplements of vitamin E in the form of $\alpha$TAc as follows: 0.5mg (control group), 100mg (E-excess group). The third group (E-deficient group) received no $\alpha$TAc. After the animals had been killed by decapitation, the livers were removed and weighed. An aliquot of the liver was homogenized and extracted, using the method of Ogur and Rosen (2), and DNA was determined according to the procedure of Burton (3).

RESULTS AND DISCUSSION

Table I shows the effect of $\alpha$TAc, and of deprivation of tocopherol in the young rat. It is seen that the E-deficient animals fail to grow normally, compared with the control and E-excess groups. The striking weight loss of the E-deficient animals is obviously due to loss of appetite, for they practically ceased eating, apparently because of the unpalatability of the very low fat
diet. On the other hand, when αTAc was added as a daily supplement, whether in normal or excess amounts, appetites were restored and they grew at a substantially normal rate.

The liver weights, to a considerable extent, paralleled general growth rates, and it is seen that the ratio of body weight to liver weight is essentially constant, irrespective of whether the animal is deprived of vitamin E or not.

No influence of vitamin E deficiency was seen in the DNA content per unit weight of liver; however, the administration of αTAc in excess amounts significantly increased the DNA content when compared with the control or vitamin E-deficient animals. Assuming a constant amount of DNA per mean cell nucleus, the average cell number can be calculated. Similarly the cell mass in grams, which is 1/cell number, can be computed. From these calculations (Table I), it is evident that the number of cells per gram of liver tissue is significantly greater in rats fed an excess of αTAc. As a corollary, the cell mass is significantly smaller.

Several workers have studied the relationship between vitamin E and the nucleic acids, using the classical approach of inducing a characteristic deficiency in the experimental animal, and deducing vitamin function by observing the changes in comparison with non-deficient animals. Thus Dinning, Sime and Day (4) found, in the rabbit, that vitamin E deficiency fails to affect the incorporation of C\textsuperscript{14}-labeled formate into DNA of liver tissue, although there is extensive incorporation of C\textsuperscript{14}-labeled guanylic acid (5); P\textsuperscript{32} is incorporated into nucleic acids of all tissues studied, including liver, at considerably increased rates in E deficiency (6). In E-deficient rats (7) the incorporation of C\textsuperscript{14}-labeled formate into nucleic acids is twice that of normal controls. These findings were interpreted as an indication that “regulation of the turnover rate of nucleic acids is believed to be a primary metabolic function of vitamin E”. In spite of the increased turnover rate in E deficiency, the concentration of nucleic acids remains unchanged (8). Other workers have found that the daily administration of small amounts of αTAc to normal rats fails to modify the amount of DNA in liver, but if the supplement is increased to 100 mg per day a significant increase in the DNA occurs (9). Niesar (10) found that the infusion of tocopherol in Tween 60 into a neck vein of normal rats leads to significant increases in DNA of liver as well as in other tissues.

Our findings agree with the general body of scientific opinion on this matter. Vitamin E deficiency does not alter the concentration of DNA, per unit wet weight of rat liver, despite drastic changes in body and liver weight, but the administration of massive doses of αTAc results in significantly higher levels of liver DNA in the rat. It is recognized that polyploidy in liver complicates any attempt to assign a constant amount of DNA per cell. Therefore any value obtained from organ analysis is necessarily an average value. Nevertheless, other workers who have studied this phenomenon in some detail have concluded that a mean cell content of DNA is the most stable and reliable parameter of the cell (1). The increased amounts of DNA found in
liver following administration of $\alpha$TAc can be referred to the cellularity of the tissue, assuming a mean cell amount of $9.3 \times 10^{-12}$ g from which the cell mass can be calculated (Table I). It is apparent, therefore, that $\alpha$TAc in large doses, in the rat, stimulates hyperplasia in the liver, and it follows that the cell mass is correspondingly reduced.

### Table I

**Influence of Dietary $\alpha$-Tocopheryl Acetate on Mean Body Weight, Liver Weight, Deoxyribonucleic Acid, Cellularity and Mass of Rat Liver**

<table>
<thead>
<tr>
<th></th>
<th>Control $(N=6)$</th>
<th>E-deficient $(N=6)$</th>
<th>E-excess $(N=6)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>105.6 ± 4.6</td>
<td>53.5 ± 4.4</td>
<td>105.6 ± 3.5</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>4.77 ± 0.43</td>
<td>2.58 ± 0.10</td>
<td>5.27 ± 0.34</td>
</tr>
<tr>
<td>Body weight</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Liver weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA per liver (mg)</td>
<td>4.88 ± 0.37</td>
<td>2.87 ± 0.23</td>
<td>7.17 ± 0.52</td>
</tr>
<tr>
<td>DNA per g (mg)</td>
<td>1.03 ± 0.07</td>
<td>1.11 ± 0.06</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>Cellularity per liver ($\times 10^6$)</td>
<td>524 ± 41</td>
<td>309 ± 24</td>
<td>771 ± 55</td>
</tr>
<tr>
<td>Cellularity per g ($\times 10^6$)</td>
<td>110 ± 7.5</td>
<td>120 ± 6.5</td>
<td>146 ± 5.3</td>
</tr>
<tr>
<td>Cell mass ($\times 10^{-9}$ g)</td>
<td>9.1 ± 0.58</td>
<td>8.6 ± 0.35</td>
<td>6.6 ± 0.48</td>
</tr>
</tbody>
</table>

Control rats were fed 0.5 mg $\alpha$-TAc per rat per day. E excess rats were fed 100 mg $\alpha$-TAc per rat per day. $N$ is the number of animals.

In previous experiments, rats maintained on a diet of rice, bran and meat mash, when fed large amounts of $\alpha$TAc (11) did not show any effect of the vitamin on DNA concentration in liver. However, these animals were not weaning rats, and it has been amply demonstrated that the most pronounced effects of vitamin E deprivation or administration are to be seen in young animals.
SUMMARY

1. Male weaning rats were divided into three groups and maintained for several weeks on a low fat, vitamin E-free diet.
2. Two of the groups termed control and E-excess groups were given, respectively, daily supplements of 0.5 mg and 100 ml $\alpha$-tocopherol.
3. The third group termed E-deficient received no $\alpha$-tocopherol.
4. The E-excess group had significantly higher concentration of DNA in liver than the control or E-deficient groups.
5. On the basis of an assumed constant amount of DNA per cell, it has been possible to compute the number of cells per unit weight of tissue and per liver, as well as the cell mass.
6. It is concluded that $\alpha$-tocopherol in excess amounts stimulates hyperplasia in rat liver in the young animal.

ACKNOWLEDGEMENTS

The authors are indebted to Hoffman-La Roche and Co. Ltd. Basle, Switzerland, for a generous gift of DL-tocopheryl acetate (synthetic vitamin E), to the Research Grants Committee of the University of Hong Kong for supporting this work, and to the China Medical Board of New York, Inc., for their generous gifts of equipment used in this research.

REFERENCES