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THE PROBABLE HAZARDS OF SUCROSE IN MAN

H. Mohammadiha*

ABSTRACT

The effect of dietary sucrose on DNA formation in rat liver and kidney compared with that of starch was studied. Male, weanling rats were fed for six and 14 days on diets containing 68% sucrose or 68% starch, and the liver and kidneys were examined for the amount and rate of formation of DNA, and also for content of fat, protein, and moisture. Food intake and weight gain were estimated.

Sucrose fed animals had heavier liver and kidneys than starch fed animals. Food intake and weight gain were similar on both diets. The enlargement of the liver after six days was mainly due to increase in cell number calculated from DNA content. Whereas after 14 days the enlargement was mostly due to increase in cell size. The proportion of protein and moisture in the liver of sucrose fed animals was less than in the starch fed ones, whereas fat content was 25% higher. Sucrose feeding had no effect in six days on weight of kidneys, but after 14 days heavier kidneys were produced.

Examination of cortex showed no difference in cell number or cell size. Twenty-four hours before killing, the animals were injected intraperitoneally with H^3 thymidine. Radioactive count was too low, the results too variable for any conclusions to be drawn.

INTRODUCTION:

There are many reports in the literature indicating that the various dietary carbohydrates differ in their physiological effects. Nearly a century ago, Frerichs, (1876(1), Kulz, 1876(2), and Lusk, 1892(3) fed fasted animals on various carbohydrates and concluded that the effects of sucrose differed from those of starch. Many investigators have reported that the liver weights and sometimes the kidney weights of sucrose-fed rats have been found to be greater than those of rats fed with starch. (4), (5), (6), (7).

Sucrose feeding experiments were conducted using rats to determine :

* Department of Biochemistry, Univedsity of Teheran, Iran.

1. Whether the resultant enlargement of the liver and kidneys was due to an increase in cell size (hypertrophy) or to an increase in cell number (hyperplasia) or both; 2. If the rate of formation of DNA is also affected; and 3. Whether the composition of liver (protein, fat and water) is changed.

Experimental:

General Methods:

Animals were fed on sucrose or starch for six days in the first experiment and 14 days in the second experiment. Liver and kidneys were analysed and the DNA content determined. The rate of DNA formation was measured by injecting labelled thymidine 24 hours before killing.

Animals:

Thirty two male weanling rats (of sprague Dawley strain of 21 days of age obtained from Animal Suppliers Ltd.) were used. The four litters each of eight rats, weighed 44-99gm. at the start of the experiment. This unusually wide range in body weight of rats of the same age is commented upon later.

Before the experiment the animals were fed on the cube stock diet for a period of one week, so they were 28 days old at the beginning of the experiment. They were housed in galvanised iron cages (one rat in each cage) with raised grids of 3/8" mesh and maintained at a temperature of 22-23°C. They were divided into 4 groups: two experimental (sucrose-fed) and two control (starch-fed); one animal from each litter was put in to each group so that results from each rat could be compared with those of their litter mates. Weights were matched as far as possible. Two groups of animals were fed for six days (one on sucrose, one on starch) and 2 groups for 14 days.

Diets:

Diets were composed of 68.2% sucrose or starch, 23%, case in, 0.8% arachis oil and 4% vitamin mixture, and 4% mineral mixture. Animals were allowed free access to food until the time of killing. Food intake was measured every 2 days; (Spilt food was collected on sheets of paper on the bottom of the cage).

Examination of Tissues:

Twenty four hours before killing with chloroform the rats were injected intrapritoneally with 0.25 ml H^3-thymidine (Radiochemical Centre) containing 7.35 μ C. $H.^3$. After death the liver and kidneys were remover. The organs were weighed immediately and stored frozen for DNA, crude protein, moisture and fat estimation. The incorporation of H^3-thymidine into DNA was measured as total radioactivity (counts/mg. tissue/min.).

Fat extraction and estimation :

One gm. of liver was homogenised with 5 ml. of choloroform methanol (2:1) in a glass potter-Elvehjem homogeniser and the homogeniser tube wase washed twice with, up to 5 ml. Solvent. The homogenate was filtered into a separating funnel, and filtrate was washed with water according to the method of Folch, Lees and Stanley, 1957 (8).

Estimation of Deoxyribonucleic Acid (DNA) :

One gm. of liver (or 0.5 gm. of kidney cortex) were homogenised with 9 ml. (or 4.5 ml.) of distilled water. One ml. of 10% homogenised tissue (duplicate test) with 5 ml. of cold 10% trichloroacetic acid (TCA) were mixed and centrifuged, and the supernatant was discarded. The residue was re-suspended in 5 ml. of 0.5 N perchloric acid (PCA) and re-centrifuged. This process was repeated twice more. The tubes were kept cold up to this stage to prevent the breakdown of nucleic acids to acid soluble fragments. The insoluble residue was resuspended in 0.5 N PCA, and nucleic acid was extracted by heating in a water bath at 96 $\pm$ 1°C. for 10 minutes. The contents were then cooled and centrifuged, the supernatant was poured into a centrifuge tube and the residue further re-extracted with PCA. The two solutions were combined and the volume was adjusted to 5 ml. with 0.5 N PCA. This procedure is a modification of that of Wannemache et al. 1965(9), using PCA, instead of TCA, omitting the fat extraction and reducing the time of heating with PCA from 45 to 10 minutes. This modification gives more consistent and higher results. The estimation makes use of colorimetric reaction of deoxyribose with diphenylamine reagent (Burton 1965 (10)). All tests were duplicated and the standards of pure DNA, calf thymus DNA (Mann) in 0.3 N KOH in 10 ml. was carried out simultaneously under the same conditions (50, 100, 150, 200, 300, 400, μ g/2 ml. of standard).

Measurement of Radioactivity:

The method of Winick and Noble, 1965 (11) was used in which 0.5 ml. of 1/10 homogenised liver with 2 ml. Soluene (Packard Soluene TM 100 Sample solubilizer : (12)) was mixed and kept at 37°C. over night to dissolve the tissue. Then eight ml. of 0.8% PBD (Scales, 1967 (13)) in toluene were added, and the sample was counted in a Packard liquid scintillation counter. All samples were duplicated and were corrected for quenching using the counting efficiency versus channel ratio for H^3. Radioactivity was calculated both as count per minute per mg. liver and as count per minute per whole liver.

Moisture : Moisture was determined by drying at 105°C. overnight.

Nitrogen content : Nitrogen content was determined by semi-micro Kjeldahl, (14), (15) and crude protein was calculated by N X 6.25. Energy

value of foodstuffs for both diets was determined in the ballistic bomb colorimeter (Miller and payne, 1959 (16)). There was a small difference in energy value between the diets (starch diet 1 gm. = 17.68 KJ (4.23 Kcal) and sucrose = 17.36 KJ (4.16 K cal.)).

Statistical Analysis:

All statistical comparisons were tested for significances of the differences using non-parametric Wilcoxon Match-pair signed-ranks test (17) for litter mates.

Results:

The average of the initial and final weights, the food intake, weight gain and food conversion efficiency (Food eating/g. Weight gain) was similar in both experiments. (table I & II).

Although the animals were purchased as 21-day old litter mates the wide range in body weights at the beginning of the study was suspected to be due to age difference. So far as possible animals were matched on the basis of litters and body weights but the possible age differences may have had some effect on the results.

In comparison with Starch-fed rats, the liver weight (expressed as a percentage of body weight) of the sucrose-fed animals increased by 11% and 16% after six and 14 days feding respectively. The fat content of the liver increased by 8% and 26% respectively, (tables III & IV).

After six days the cell number in liver calculated from DNA content had increased by 8% (P = 0.016). The cell size remained unchanged. After 14 days the cell number was the same as in starch-fed rats but the cell size had increased by 8% (P = 0.020), (tables V & VI).

Radioactivity counts showed very large variations betwen samples so that although the livers of sucrose-fed rats after 14 days gave a 20% higher count than the livers of starch-fed rats, the differences was not significant. The results were not acceptable because of the low count relative to background count, e.g. in many instances 150 counts/minute, compared with a background count of 40-70 minute.

The weight of the kidneys, amount of DNA per gram of kidney's cortex, the number and size of cells were also measured. Sucrose feding for six days had no effect on these measurements but after 14 days produced 9% (P = 0.015) heavier kidneys (calculated per 100 g. body weight), (tables VII & VIII).

Examination of the cortex showed no difference in cell number or cell size. Calculation of the number and size of the cells is based on the assumption that DNA content of the nucleus is constant. If it is also assumed that rat tissues are made up of cells with diploid nuclei, then the weight of DNA in the average cell calculated by the formula of Enesco, 1962 (18) is 6.2 pg.

The hepatic cells of the rat, however, are known to contain polyploid cells, and there is an increase in binucleated cells with age (11), but as the aim of this work was a comparison of the effects of different carbohydrate on size and number of cells, the calculations were based on the figures of 6.2 μg.

GENERAL DISCUSSION:

In the first experiment (six days), the increase of 11. % in liver weight was largely or entirely due to the increase in cell number (8%). The results after 14 days feeding are not so clear-cut since three of the eight rats grew poorly and did not have heavier livers than their litter mates. If the results from all eight rats are combined the 15.6% increased liver weight could be accounted for by an increase of 0.5% in cell number (not statistically significant), and an increase of 9% ($P = 0.02$) in cell size. Although it is not possible to calculate significance by the method of Wilcoxon (17) on only 5 animals, when the abnormal rats are omitted from consideration a 22% increase in liver weight is observed, which is accounted for by an approximately equal increase in cell size and cell number (hypertrophy and hyperplasia).

The liver protein per 100 g body weight was less in sucrose-fed rats (7.6% in 6 days feeding and 8.75% in 14 days feeding) than in starch-fed rats; although this was not statistically signficant after 6 days feeding it was significant after 14 days ($P = 0.036$) (tables III & IV).

It has ben shown by many workers that dietary sucrose increases the fat synthesis and the level of fat in liver (Bender, 1971 (19), Shiff, 1971 (20) and Bender et al, 1972 (21)), however, others (Rombery and Denton, 1965 (22)) found that dietary sucrose reduced the food intake and resulted in less body fat, more nitrogen and water. In the present investigation the amount of liver fat increased by 25% by sucrose feeding, and consequently the moisture decreased.

In investigations of the toxicity of food additives, liver enlargement was, at one time, taken as a sign of toxicity. It might, however, be regarded as the result of hypertrophy. It is possible, although there is no evidence, that the enlargement of the liver induced by sucrose might be a benefit to the animal if the enlargement is due to an increase in actively metabolising tissue. Ismail (23) found that rats fed on sucrose were less susceptible to anaesthetic, Nembutal, than those fed on starch-they woke earlier. He offered no explanation other than the possibility of an increase in detoxicating enzymes. Dickerson et al, (24) (25), found no increase in the four detoxicating enzymes that he examined. However, the detoxication of Nembutal might depend upon other enzymes whose activity after feeding sucrose has not been measured.

Clearly, the increased liver size, whether in cell size or cell number should be further investigated to examine, in particular, whether there is a corresponding increase in the metabolic activity of the organ.

The enlargement of the kidneys also requires further examination.

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TABLE I

FOOD INTAKE, WEIGHT GAIN AND EFFICIENCY OF DIET

(6 DAYS EXPT.)

	Starch	*Sucrose*
Initial weight (g.)	69.8 (63-79)	68.4 (64-90)
Final " (g.)	103.2(90-126)	102.6 (94-113)
Weight gain (g.)	33.7 (27-41)	34.0 (27-37)
Food intake (g.)	65.9 (52-76)	64.7 (59-74)
Food eaten/g. wt. gain	1.96 (1.86-2.1)	1.92 (1.75-2.1)

TABLE II

FOOD INTAKE, WEIGHT GAIN AND EFFICIENCY OF DIET

(14 DAYS EXPT.)

	Starch	*Sucrose*
Initial weight (g.)	66.6 (52-91)	65 (44.90)
Final " (g.)	143 (126-178)	138 (110-165)
Weight gain (g.)	77.0 (67-94)	73.0 (64-81)
Food intake (g.)	189.6 (177-202)	177.2 (154-195)
Food eaten/g. wt gain	2.46 (2.12-2.60)	2.48 (2.22-3.02)

TABLE III

LIVER COMPOSITION AFTER 6 DAYS FEEDING
(28 to 34 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*	*Sucrose/ Starch*
Liver weight (g.)	5.28 (4.31-6.16)	0.062	5.82 (4.77-6.92)	1.102
" " as % body weight	5.09 (4.38-5.60)	0.01	5.66 (5.13-6.00)	1.111
Crude protein mg/whole liver	921 (820-1074)	N.S.	986 (865-1144)	1.070
" " g/ liver	17.5 (16.0-18.5)	N.S.	17.0 (15.7-18.3)	0.971
Fat g % liver	4.60 (4.20-5.0)	0.024	5.0 (4.4-6.5)	1.086
" mg/whole liver	242.8 (208.3-291.8)	0.034	290.1 (241.3-388)	1.194
Water %	70.8 (69.9-71.46)	N.S.	70.0 (67.0-72.0)	0.988

TABLE IV

LIVER COMPOSITION AFTER 14 DAYS FEEDING
(28 to 42 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*	*Sucrose/ Starch*
Liver weight (g.)	6.78 (6.03-8.17)	0.05	7.6 (5.55-9.17)	1.120
" " as % body weight	4.73 (4.6-4.79)	0.01	5.47 (4.98-6.32)	1.156
Crude protein mg./whole liver	1243 (965-1477)	N.S.	1300 (1090-1330)	1.045
" " g. %	18.26 (17.2-18.8)	0.036	17.17 (16.4-18.6)	0.940
Fat g % liver	5.0 (4.60-5.20)	0.02	6.28 (5.10-6.90)	1.256
Fat mg./whole liver	538 (289-408)	0.024	4.80 (309-779)	1.420
Water %	70.87 (70.4-71.20)	0.048	69.64 (68.3-71.30)	0.982

TABLE V

LIVER CELL SIZE AND NUMBER AFTER 6 DAYS FEEDING
(28 to 34 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*	*Sucrose/ Starch*
DNA mg./g. liver	5.21 (4.91-5.62)	N.S.	5.08 (4.66-5.97)	0.975
Total DNA/whole liver	27.15 (24.2-29.8)	N.S.	29.44 (27.9-32.3)	1.084
Cell number X 10^9	4.380 (3.91-4.82)	0.016	4.749 (4.80-4.879)	1.084
Cell mass. Pg.	1.22 (1.10-1.32)	N.S.	1.22 (1.03-1.32)	1.000
C.P. m./mg. liver	41.95 (20.97-82.10)	N.S.	42.66 (15.98-75.35)	1.016
C.P. m./whole liver X 10^3	212.6 (97.1-354.1)	N.S.	237.1 (110.4-449.8)	1.115

TABLE VI

LIVER CELL SIZE AND NUMBER AFTER 14 DAYS FEEDING
(28 to 42 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*	*uScrose/ Starch*
DNA mg./g. liver	5.23 (4.9-5.6)	0.02	4.70 (4.3-5.4)	0.898
Total DNA/whole liver	35.45 (31.5-37)	N.S.	36.26 (28.4-42.99)	1.022
Cell number X 10^9	5.718 (5.091-6.815)	N.S.	5.842 (4.585-6.934)	1.021
Cell mass pg.	1.18 (1.10-1.25)	0.02	1.28 (1.14-1.30)	1.084
C.P. m./mg. liver	24.62 (16.96-30.44)	N.S.	29.65 (12.97-47.63)	1.204
C.P. m./whole liver X 10^3	165.0 (123.6-230.9)	N.S.	220.7 (113.9-353.7)	1.337

TABLE VII

KIDNEY COMPOSITION AFTER 6 DAYS FEEDING
(28 to 34 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*
Weight of kidneys (g.)	1.083 (0.892-1.192)	N.S.	1.091 (0.944-1.273)
" " /body weight	1.049 (0.910-1.180)	N.S.	1.065 (1.012-1.163)
DNA mg./g. cortex	11.32 (10.83-12.22)	N.S.	11.241 (10.83-12.22)
Cell number X 10^9	1.91 (1.59-2.18)	N.S.	1.93 (1.73-2.21)
Cell mass, pg.	0.54 (0.50-0.57)	N.S.	0.54 (0.50-0.57)

TABLE VIII

KIDNEY COMPOSITION AFTER 14 DAYS FEEDING
(28 to 42 DAYS OF AGE) (MEAN AND RANGE)

	Starch	*P Value*	*Sucrose*	*Sucrose/ Starch*
Weight of Kidneys (g.)	1.594 (1.414-1.999)	N.S.	1.664 (1.353-1.925)	1.043
" " /body weight	1.109 (1.048-1.446)	0.015	1.214 (1.079-1.418)	1.094
DNA mg./g. cortex	20.41 (18.33-20.83)	N.S.	19.37 (17.77-20.83)	.949
Cell number X 10^9	3.65 (3.32-3.91)	N.S.	3.79 (3.38-4.57)	1.038
Cell mass, pg.	0.312 (0.28-0.38)	N.S.	0.318 (0.30-0.39)	1.019

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