

# Effect of Different Levels of Protein in Sucrose and Starch Diets on Growth and Liver Deoxyribonucleic Acid (DNA) in the Young Rat

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M. Akmal Khan \*

and

A. E. Bender \*\*

**Abstract:** *The influence of the type of dietary carbohydrate on growth, liver weight and liver DNA of growing rats fed ad libitum the experimental diets containing 7, 10, 15 and 20 per cent protein for 91 days was studied. The body weight and liver weight of rats fed either starch or sucrose increased with the increasing levels of protein in the diet. The groups on starch grew faster than those receiving sucrose when the levels of protein were 7, 10 and 15 per cent. However, the difference between the two carbohydrates disappeared when protein content was increased to 20 per cent. Sucrose produced heavier livers and a greater total DNA content than did starch. The number and size of the cells were calculated and it was established that the increase of liver size induced by dietary substitution of sucrose for starch was caused by an increase in the number of cells.*

## INTRODUCTION

There is conflicting evidence of the effects of dietary carbohydrates on animals. Various investigators have demonstrated that under certain experimental conditions cornstarch supports better growth than does sucrose (Monson et al.<sup>12</sup>, Harper and Katayama,<sup>6</sup> and Harper et al.<sup>7</sup>). Spivey et al.<sup>14</sup> indicated that improvement in the efficiency of protein utilization when starch is substituted for sucrose is too small to account for the apparent protein-sparing effect of starch.

Sucrose in the diet of rats leads to production of livers heavier than those of rats fed diets with starch (Bender and Damji<sup>1</sup>, and Khan<sup>9</sup>). The effect of different levels of protein in sucrose and starch diets on growth and liver DNA to determine whether the increase in liver size induced by sucrose is due to an increase in cell size or to an increase in cell number or to both is reported in this paper.

## MATERIAL AND METHODS

Fifty six young male, Sprague-Dawley six weeks old rats were divided randomly into eight groups of seven animals each and were fed ad-libitum the experimental diets (Table 1) containing 7, 10, 15 and 20 per cent protein for 91 days. The temperature in the animal rooms was maintained at 70-72°F.

At the end of the experiment the rats were killed by a blow on the head and the livers were rapidly removed, rinsed in ice cold saline, dried on filter paper, and weighed. One gram of the liver was homogenized with 9 ml 0.15 NaCl in 0.2 M Tris buffer pH 9.6 at 0°C.

DNA was estimated by the colorimetric method (Volkin and Cohn)<sup>15</sup>. For true protein (N X 6.25) homogenate was precipitated with 10 per cent trichloroacetic acid and nitrogen was determined by micro-kjeldahl method.

\* Department of Nutrition, University of Agriculture, Lyallpur.

\*\*Department of Nutrition, Queen Elizabeth College, University of London, London.

**Table 1: Composition of Experimental Diets**

Ingredients	A	B	C	D	E	F	G	H
Casein (Casumin)	7.0	7.0	10.0	10.0	15.0	15.0	20.0	20.0
Corn starch	85.0	15.0	82.0	12.0	77.0	7.0	72.0	2.0
Sucrose	—	70.0	—	70.0	—	70.0	—	70.0
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mixture	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The data were subjected to statistical analysis by using Mann Whitney U test (Mann and Whitney<sup>10</sup>) and Jonckheere Test (Jonckheere<sup>8</sup>).

### RESULTS AND DISCUSSION

Average body weight, liver weight and liver DNA of rats fed 7, 10, 15 and 20 per cent protein diets with either starch or sucrose are given in Table 2.

**Table 2 : Effect of Starch and Sucrose Diets on Body Weight, Liver Weight and Liver (DNA) Content of Rats Fed Different Levels of Protein (Mean Values)**

Dietary protein levels (%)	Dietary Carbohydrate	Initial weight g	Final weight g	Liver weight g	Liver Wt. mg/g body Wt.	DNA g/100 liver	DNA mg/total liver	DNA mg/g true protein	Cell No. x10 <sup>9</sup>	Cell mass pg
7	Starch	88	277	8.8	3.1	3.4	30.3	23.0	4.9	1.8
	Sucrose	91	238	8.7	3.7	3.5	30.7	23.1	5.1	1.9
10	Starch	88	361	10.7	2.9	3.5	37.9	21.9	6.1	1.9
	Sucrose	92	349	12.8	3.6	3.3	42.4	21.6	6.8	1.8
15	Starch	86	420	12.6	3.0	3.5	43.2	20.5	6.9	1.8
	Sucrose	85	384	13.6	3.5	3.4	45.8	20.1	7.4	1.9
20	Starch	86	420	13.6	3.2	3.4	46.4	19.9	7.4	1.8
	Sucrose	89	415	15.3	3.7	3.4	51.8	20.2	8.4	1.8

### GROWTH

The mean growth rate of the rats on different diets is illustrated in Fig. 1.

The body weights of rats fed either starch or sucrose as a source of dietary carbohydrates increased significantly ( $P < 0.001$ ) with the increasing levels of protein in the diet.

With 7 per cent protein diet the groups fed starch grew significantly ( $P < 0.05$ ) faster than those fed sucrose. When the level of protein in the diet was increased to 10 per cent the rats fed starch and sucrose gained on average 273 and 257 gms respectively and the difference was not significant. The mean gain in body weights on starch and sucrose diets containing 15% protein was 334 and 299 gms respectively and the difference was significant ( $P < 0.004$ ). There was no significant difference in growth rates of the rats fed 20 per cent protein diet with either starch or sucrose as a source of dietary carbohydrate.

The food intake was not measured in this experiment. The better growth on starch could be attributed to the increased food intake. Harper and Katayama<sup>6</sup> found that rats receiving the corn starch ate more than those receiving sucrose and they fur-

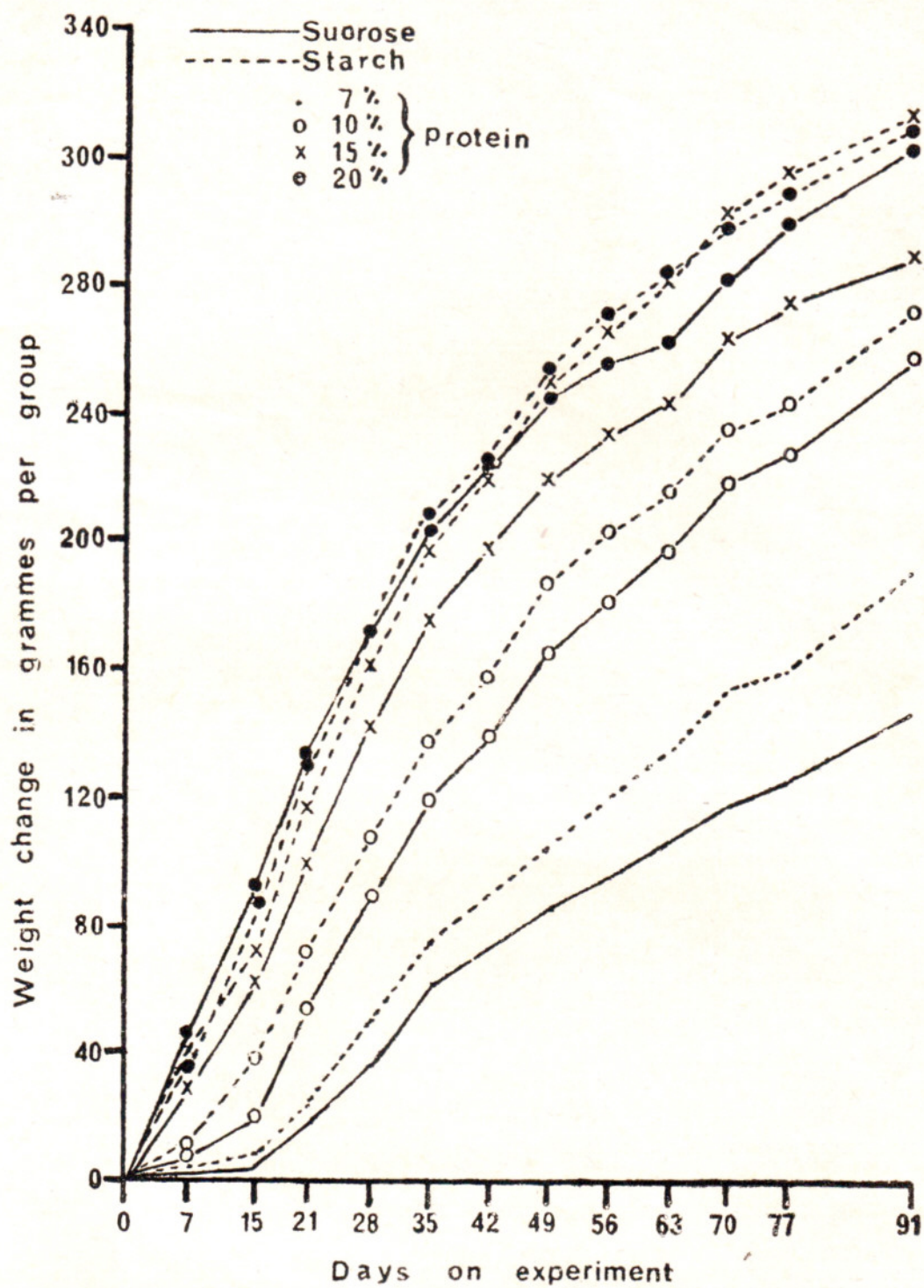


FIG. 1 GROWTH OF MALE RATS.

Effect of protein sucrose and starch on rat liver DNA

ther supported the present findings that rats receiving 9 per cent casein diet grow better with cornstarch than with sucrose but no marked differences were noticed between groups of rats fed 18 per cent casein with either of these carbohydrates.

It is well known that most starches contain small amounts of protein and it might be suggested that the low casein diet is supplemented by the amino acids from the protein in the starch. Monson *et al.*<sup>12</sup> have shown that dextrin rations pass through the gastrointestinal tract of the chick more slowly than do sucrose rations. In work with rats Geiger<sup>5</sup> has suggested that carbohydrate fed with protein reduces the rate of deamination of amino acids in the liver. Womack *et al.*<sup>16</sup>, Marshall and Womack<sup>11</sup> and Khan<sup>9</sup> have demonstrated increased nitrogen retention in rats fed low protein diets in which sucrose was replaced with corn starch. It is suggested that the slower rate of digestion and slower passage of starch along the intestine with the consequently more continuous absorption compared with that of sucrose, may provide a protein-sparing effect.

#### LIVER WEIGHTS

The weights were expressed both as gm and gm/100 gm body weight. The weight of livers of animals fed either starch or sucrose increased with the increase of protein levels in the diet. Animals fed sucrose diets containing 7, 10, 15 and 20 per cent protein had significantly ( $P < 0.007, 0.001, 0.002$  and  $0.001$  respectively) heavier livers (gm/100 gm body weight) than rats fed starch. (Fig. 2).

The livers were not analysed for fat, but it is unlikely that the weight difference was due to fat. The factors governing the liver fat response to sucrose feeding may be age, sex as well as strain of animals, the protein concentration and duration of the experimental period. Harper *et al.*<sup>7</sup> found no difference between the liver fat of starch and sucrose with 9 per cent casein fed rat after 12 weeks. Bender and Thadani<sup>2</sup> fed 24 per cent protein diet with starch or sucrose a source of carbohydrate to three different strains of rats and observed no difference between the liver fat of two groups.

The heavier livers on sucrose in the present experiment may be due to:

1. Increase in the number of cells (hyperplasia) or
2. enlargement of cells (hypertrophy) or
3. both types of responses may be combined.

#### LIVER DNA

When DNA concentration was expressed as mg per gm of liver or per gm of true protein, no difference was observed between animals fed either of carbohydrates at high levels of protein. Since, however, sucrose produced heavier livers than did starch, total liver DNA was increased by sucrose. This increase was significant with 10 per cent ( $P < 0.027$ ) and 20 per cent ( $P < 0.036$ ) protein but was not significant with 15 per cent protein, possibly, because there was an unusually high variation in the results.

A diet with sucrose and 7% protein resulted in a significant reduction in weight gain of the rats. This accounts for the fact that, although sucrose produced livers that weighed less in absolute terms, they were heavier when expressed per 100 g body weight. Similarly, the total DNA and the total number of cells did not differ in livers of rats fed either sucrose or starch, but when expressed in terms of body weight the livers of the sucrose fed rats had more DNA and thus a greater number of cells. Total liver DNA per 100 g body weight was 133 mg with sucrose and 108 mg with starch.

The number of nuclei or cells was calculated from the total liver DNA in mg  $\times 10^9$  divided by 6.2 (Enesco<sup>3</sup>). Cell mass was calculated from the total liver weight divided by the number of nuclei. There were more cells in the liver of rats fed sucrose diet at all levels of protein. No increase in the cell mass was observed on sucrose diet.

Calculation of the number and size of the cells is based on the assumption that the DNA content of the nucleus is constant, and the amount was established for rat tissues as 6.2 pg DNA per nucleus corresponding to diploid nucleus (Enesco<sup>3</sup>). The hepatic cells of the rat, however, are known to contain polyploid cells and there is an increase in binucleated cells with age (Enesco and Leblond<sup>4</sup>, Smith and Copenhauer<sup>13</sup>). Calculations based on the figure 6.2 pg will thus not give correct absolute values. The intention of this work, however, was not to determine absolute cell

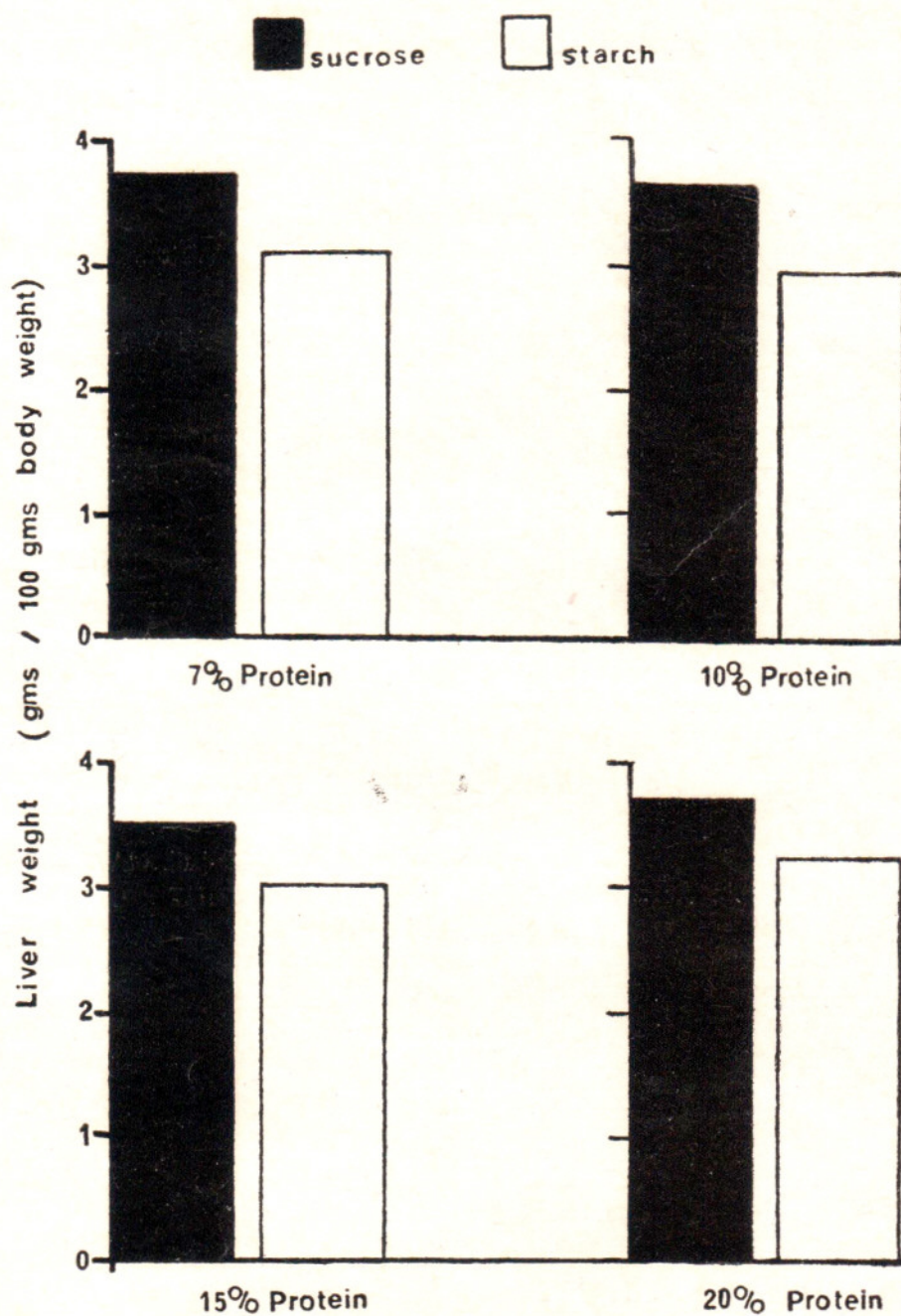


FIG. 2 LIVER WT OF GROWING RATS AFTER 91 DAYS ON DIETS

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size or number but to compare these values after dietary changes. On this basis it is established that the enlargement of liver observed after feeding sucrose is due to an increase in the number of cells rather than an increased cell size.

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