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Sucrose Induction of Hepatic Hyperplasia in the Rat

SUCROSE in the diet[1] of rats leads to production of livers heavier than those of rats fed diets with starch[2]. We report here experiments to determine whether the increase in liver size induced by sucrose is due to an increase in cell size (hypertrophy) or to an increase in cell number (hyperplasia) or to both.

Male Sprague–Dawley rats were fed on a stock laboratory diet from the age of 4 weeks, until they weighed 200–230 g at 60–70 days old. They were then placed onto the purified diets described below. Water and food were given freely during the course of the experiments. At the end of the experiment the rats were killed, 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 M NaCl in 0.2 M Tris buffer, *p*H 9.6, at 0° C.

DNA was determined by the method of Volkin and Cohn[3]. Trichloroacetic acid was added and the amount of true protein in the precipitate produced was determined by the micro-Kjeldahl method.

Results are reported as significant when $P < 0.05$, as calculated by the Mann–Whitney[4] method.

In the first experiment five groups, each of six rats, were put on diets in which the carbohydrate was corn starch, sucrose, fructose, glucose or maltose. The composition of the diets was carbohydrate 70%, casein 24%, arachis oil 0.8%, salts 4%, 'solka-floc' cellulose 2% and vitamin mixture[5]. After 30 days on these diets, the animals were killed and the livers examined.

Table 1 Effect of Dietary Carbohydrates on Rat Liver. Median Values, 6 Rats in Each Group

Dietary carbohydrate	Liver weight g	Liver weight g/100 g body weight	DNA mg/g liver	DNA mg/g protein	Cell No. $\times 10^9$	Cell mass, pg
Starch	11.1	3.5	2.58	17.2	4.4	2.41
Sucrose	13.6 * †	3.8 * †	2.63	17.5	6.3 * †	2.36
Fructose	15.3 * †	4.3 * †	2.13 * †	15.7 *	5.2 * †	2.91 * †
Glucose	11.4	3.3	2.41	16.4	4.4	2.56
Maltose	12.4	3.4	2.49	15.7	4.8	2.44

* Differs significantly from starch.
† Differs significantly from glucose.

The livers of rats fed on diets with starch, glucose or maltose showed no significant differences in weight or DNA content (Table 1). Sucrose and fructose produced heavier livers and a greater total DNA content than did starch, glucose or maltose. With sucrose, there was no difference in DNA content when it was calculated in terms of unit liver weight or protein, indicating that there was an increase of about 40% in cell number, but no change in cell size.

With fructose, there was an increase in total DNA, but a decrease in terms of unit liver weight. This suggests that fructose produced an increase both in cell number and in cell size.

In the second experiment, eight groups, each of seven rats, were given diets with casein contents of 7%, 10%, 15% or 20%, and either starch or sucrose as the major carbohydrate. Diets were made up as in the first experiment, except for the proportion of carbohydrate and casein. The sucrose diets were 70% sucrose, the remainder being made up with starch. The starch diets contained 72–85% starch to compensate for the reducing protein level. After 90 days on these diets the rats were killed.

Diets with the higher amounts of protein gave the same concentration of DNA in terms of unit liver weight or unit liver protein with either sucrose or starch (Table 2). Since, however, sucrose produced significantly heavier livers than did starch, total liver DNA was increased by sucrose. This increase was significant with 10% and 20% protein but was not significant with 15% protein, possibly because there was an unusually high variation in the results.

Table 2 Effects of Starch and Sucrose Diets on Livers of Rats Fed Different Levels of Protein. Median Values, 7 Rats per Group

Dietary protein level	Dietary carbohydrate	Liver weight g	Liver weight g/100 g body weight	DNA mg/g liver	DNA mg/g true protein	Cell No. $\times 10^9$	Cell mass pg
7%	Starch	9.3	3.1	3.5	22.6	5.0	1.8
	Sucrose	8.1	3.7†	3.6	23.2	4.8	1.7
10%	Starch	10.3	3.0	3.5	21.9	6.1	1.7
	Sucrose	12.4*	3.6†	3.3	21.8	6.8†	1.8
15%	Starch	12.3	2.9	3.4	20.7	7.0	1.8
	Sucrose	13.4	3.5†	3.3	20.4	7.4	1.8
20%	Starch	13.7	3.2	3.4	19.8	7.4	1.8
	Sucrose	14.6*	3.7†	3.3	20.6	8.0*	1.8

* $P < 0.05$.
† $P < 0.001$.

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.

Calculation of the number and size of the cells is based on the assumption that the DNA content of the nucleus is constant. If it is also assumed that all rat tissues are made up of cells with diploid nuclei, then the weight of DNA in the average cell[6] 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[6,7]; calculations based on the figure of 6.2 pg will thus not give correct absolute values. We were, however, less interested in the absolute number or size of cells than in the general effect on them of dietary change. We have established that the increase of liver size induced by the dietary substitution of sucrose for starch is caused by an increase in the number of cells, or at least in the number of nuclei. With fructose instead of starch, the effect is due to an increase in both cell number and cell size.

The rats in our experiment were aged 60–70 days when the experimental purified diets were introduced. It has been found that in rats fed a stock diet growth of the liver after the age of about 44 days takes place by increase in cell size only[8].

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