Comparative Ability of Certain Sugars and Honey to Enhance Saline Polydipsia and Salt Hypertension.* (31135)

C. E. HALL AND O. HALL
Carter Physiology Laboratory, University of Texas Medical Branch, Galveston

Rats fed excessive quantities of sodium chloride develop hypertensive disease(1-3). We have recently reported that if salt intake is increased by giving rats 1% saline to drink, the addition of sucrose in a 5% concentration will at least double the volume of saline consumed, and greatly accelerate the development and progression of hypertension(4-6). Such hypersalimentation was abolished by increasing sucrose concentration to 10% and was not obtained with maltose at either 5% or 10% concentration(7).

These findings were unexpected in view of the fact that in a self-selection experiment rats preferred maltose to sucrose and the latter at the higher concentration(8). Because maltose samples of different manufacture appear to be more variable in color and taste than is sucrose, it was thought desirable to repeat the maltose studies at the 5% concentration using a sugar supplied by a different manufacturer. At the same time the efficacy of d-fructose (levulose) and honey at this concentration was evaluated with respect to their ability to promote saline ingestion and enhance hypertension.

Materials and methods. Forty female rats of the Houston-Cheek strain weighing 60-70 g were anesthetized with ether, unilaterally nephrectomized and divided into 5 groups. Group 1 received 1% NaCl solution in distilled water to drink whereas Groups 2, 3, 4 and 5 were given the same solution to which honey, d-fructose (Pfanstiehl, C. P. Special), maltose (Difco, certified) and sucrose, respectively, were added to achieve a 5% concentration. Since this was an exploratory study the honey solution was prepared on a weight/volume basis without correction for the considerable amount of water contained. Preliminary observations in this laboratory indicate that maltose solutions are subject to fermentation, the gas thereby evolved causing rapid evacuation of drinking bottles, thus yielding unreliable data on fluid intake. A 1:5000 concentration of Propylparaben† was added to the maltose solution to inhibit fermentation, and to the others to equalize this factor.

Rats were individually housed in temperature-controlled quarters and given Purina laboratory chow *ad libitum*. Fluid intake was measured on 2 consecutive days each week during the experiment, and the average taken as representative of the consumption during that week. Systolic blood pressure of the animals (unanesthetized) was measured periodically by tail plethysmography, and rats with pressures above 150 mm Hg were regarded as hypertensive.

The animals were killed with ether on the 68th day and various organs were placed in neutral 10% formalin. When fixed, the organs were removed, blotted, trimmed and weighed on an analytical balance.

Results. The volume consumption of sucrose-saline was statistically greater by "Student's" test than that of saline or of any other fluid except maltose-saline ($P<0.01$ or better), in each of the 9 periods. It exceeded maltose-saline consumption ($P<.02$ or better) in all except the first, sixth and eighth periods. Maltose-saline consumption significantly exceeded that of saline ($P<.02$ or better), except in periods 2, 5 and 7. More fructose-saline than saline ($P<.025$) was consumed only in period 8. Honey did not in any period significantly influence saline consumption. The data are summarized in Fig. 1.

In terms of the speed of onset, height of blood pressure attained and number of animals involved, hypertension was more severe in the group drinking salt-sucrose solution than in any of the others until the end of the seventh week of treatment, followed in decreasing order by maltose and fructose. There was no distinctive difference between

* Supported by grants He 08054 and He 09911 from USPHS.

† Aceto Chemical Co., Inc., Flushing, N. Y.

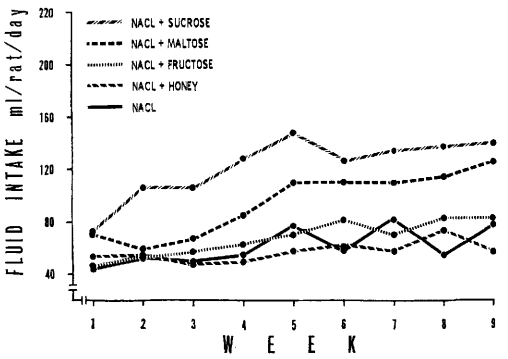

FIG. 1. Average fluid consumption of rats drinking saline with and without sweetening agents. Only sucrose consistently augmented intake above control values in each of the periods.

the group given saline and the one given honey and saline, each being less affected by hypertension than were the other groups. After the seventh week the gap between the sucrose-saline and maltose-saline groups began to narrow. This was partly because by then 4 of the 6 hypertensives in the former groups had attained pressures in excess of 214 mm Hg, and some of these thereafter began to decline, as is commonly the case when the heart begins to fail in severe hypertension. Failing health in these animals prompted the decision to abort the experiment which, if continued, would have led to increasing mortality in the group on sucrose and saline and an increasing severity of hypertension in all groups. As to the group mean systolic pressures, only that of the sucrose-saline group was ever found to differ significantly from controls which it did in each period after the first ($P<.05$ or better). Nevertheless, even though the mean blood pressures of the rats on saline did not differ significantly from those getting fructose or maltose solutions, the respective incidence of hypertension within these groups at the terminal reading was 12.5%, 67.5% and 75%, respectively. The blood pressure data are given in Table I.

An exact probability test(9) similar in purpose to the Chi-square test, but more suitable

TABLE I. Chronologic Development of Hypertension in Rats Drinking Saline or Sweetened Saline Solutions.

Exp day	Blood pressure data	1% NaCl solution	1% NaCl 5% honey solution	1% NaCl 5% fructose solution	1% NaCl 5% maltose solution	1% NaCl 5% sucrose solution
19	Blood pressure, mm Hg	124 ± 1	124 ± 1	123 ± 1	125 ± 1	126 ± 2
	% Group hypertensive	0	0	0	0	0
	% Pressures above 200 mm Hg	0	0	0	0	0
28	Blood pressure, mm Hg	125 ± 4	122 ± 1	123 ± 1	128 ± 4	149 ± 9*
	% Group hypertensive	0	0	0	12.5	50
	% Pressures above 200 mm Hg	0	0	0	0	12.5
39	Blood pressure, mm Hg	128 ± 5	123 ± 15	131 ± 8	140 ± 8	171 ± 15*
	% Group hypertensive	12.5	0	12.5	37.5	62.5
	% Pressures above 200 mm Hg	0	0	0	0	25
49	Blood pressure, mm Hg	137 ± 5	134 ± 4	151 ± 10	153 ± 7	192 ± 15*
	% Group hypertensive	25	12.5	37.5	50	75
	% Pressures above 200 mm Hg	0	0	12.5	0	50
63	Blood pressure, mm Hg	136 ± 10	141 ± 10	166 ± 16	176 ± 14	190 ± 13*
	% Group hypertensive	12.5	25	62.5	75	75
	% Pressure above 200 mm Hg	12.5	12.5	25	12.5	37.5

Systolic pressure is given as mean ± standard error.

* Significant difference from saline group ($P<.05$ or more). Whereas there is in general an increasing incidence and severity of hypertension in any group vertically with time, there is also, after the 4th week, a general trend towards increasing effect of the pure carbohydrates as one proceeds from left to right, demonstrating an increased effectiveness. Honey is ineffective.

TABLE II. Effect of Saline With and Without Added Sweeteners upon Growth and Organ Weight of Rats.

Data		1% NaCl solution	1% NaCl 5% honey solution	1% NaCl 5% fructose solution	1% NaCl 5% maltose solution	1% NaCl 5% sucrose solution
No. of rats	Initial	8	8	8	8	8
	Final	8	8	8	8	8
Body wt, g	Initial	68 ± 1*	68 ± 1	68 ± 1	68 ± 1	68 ± 1
	Final	229 ± 6	231 ± 3	234 ± 5	235 ± 5	224 ± 5
Organ wt, mg/100 g body wt	Kidney	869 ±29	834 ±50	870 ±45	788 ±16	830 ±30
	Heart	364 ±13	366 ± 2	396 ±25	405 ±15	423 ±26
	Thymus	137 ±13	139 ±10	139 ±12	137 ± 6	137 ±10
	Adrenals	33.3± 1.1	34.8± 1.0	33.6± 0.8	31.9± 1.4	31.7± 0.8

* Mean ± S.E.M.

in studies involving smaller cell frequencies, was used to evaluate significance of the findings. It showed that as compared with animals drinking saline, there was a significantly greater incidence of hypertension in those drinking sucrose-saline by the 28th day ($P = .038$) which became increasingly greater as the experiment progressed; in those drinking maltose-saline by the 39th day ($P = .019$) and in those drinking fructose-saline by the 63rd day ($P = .05$). At no time was the incidence greater in rats drinking honey-saline solution.

Further, a regression analysis was carried out in order to determine whether level of blood pressure depended upon level of saline consumption at specific points in time, without consideration of whether or not the solution was sweetened. This, of course, neglected the group structure, but it revealed correlations between the level of saline consumption and the height of blood pressure of .60, .55, .73 and .53 on days 29, 39, 49 and 63, respectively.

Organ weights revealed that heart weight, generally a reliable index of the severity of sustained hypertension, progressively increased in the sugar series proceeding from fructose through maltose to sucrose, although it just failed to achieve statistical significance even with sucrose. This prompted a statistical analysis of degree of association between heart weight and blood pressure, regardless of the fluid type consumed. The product moment correlation between heart weight and blood pressure was .89, demonstrating pronounced reactivity of heart to blood pressure elevation. This degree of association indicates that, in a population of rats varying widely in blood pressures, 89% of all variation in heart weight can be accounted for by differences in blood pressure levels. There was no significant intergroup difference in the weight of the thymus, kidneys or adrenals. Adrenals were smaller, although not significantly so, in rats drinking maltose and sucrose solutions which had shown the highest levels of salt consumption. This has been the invariable experience with sucrose-saline and is attributed to inhibition of the adrenal glomerulosa by the large quantity of NaCl consumed. However, this region, even when greatly involuted, constitutes too small a fraction of the total gland to cause uniformly a statistically significant weight reduction. Organ weights are given in Table II.

Discussion. The results with sucrose were entirely compatible with previous experience with this sugar at the 5% concentration(4-6). Whereas the response to maltose was analogous to that obtained heretofore(7) in certain respects, it differed in others. As before, the solution containing maltose was less avidly consumed than was that containing sucrose, and was less effective in accelerating the development of hypertension. On the other hand, in contradistinction to previous findings with a different maltose, significantly larger volumes of maltose-saline were generally consumed than of saline, and there was a 6-fold increase in the incidence of hypertension. The frequency of hypertension ultimately equalled that in the sucrose-saline group, although the

group pressures averaged less and the onset of hypertension was delayed. This disparity between these and the earlier experimental results lends credence to the view that maltose is not as uniform a product. Of the pure sugars fructose was least effective in promoting polydipsia and enhancing hypertension; honey was still less so, having no discernible influence upon either.

Composition of honey, depending as it does upon that of the nectars available for synthesis, is variable. Dextrose and levulose, the principal sugars, account for 65% to 80% of the bulk, and 12%-33% is water(10). Since no correction was made either for variable sugar composition or water content, the "5%" honey solution actually had a somewhat lower sugar concentration. Nevertheless, although the solution was quite sweet to the taste, the rats showed no predilection for it. This is quite surprising since levulose is slightly preferred over saline alone, and dextrose enhances saline consumption quite as strongly as does sucrose(11). The inference from this, which is supported by findings of other investigators, is that sweetness *per se* does not determine the acceptability of sugar solutions to rats.

There are other reasons for concluding that the acceptability of sugar-saline solutions to rats and hence the ability to potentiate saline polydipsia and hypertension are not clearly related to sweetness of the carbohydrates as such. If sucrose is arbitrarily accorded a sweetness index of 100, then fructose is rated at 173 and maltose at 32(10). Sucrose which has intermediate sweetness is preferred to the other two at a 5% concentration, but a preference is not evident at a 10% concentration (7). Furthermore, although rats evince a preference for saccharin(12), which to human taste is 675 times as sweet as sucrose, they are indifferent toward dulcin which is 200 times as sweet(13). Admittedly, however, the presence of salt alters the flavor of sugar solutions to human taste, and probably does so for the rat. Further experiments are in progress to delineate the properties which make certain sugar-saline solutions preferred to others and to saline by rats, but the data would suggest that in this instance neither sweetness nor calories are determinative, despite the fact that rats are said to eat primarily for calories(14).

Summary. Sucrose, maltose, fructose and honey, each at a 5% concentration in 1% sodium chloride solution used as drinking fluid, were compared in respect to their ability to influence saline consumption and the development of arterial hypertension. Honey was without detectable effect on either of these. The pure sugars, in the order of increasing effectiveness fell into the order sequence fructose, maltose and sucrose. Only sucrose significantly increased consumption of saline throughout the experiment, and was unquestionably the most effective carbohydrate in promoting saline ingestion and in enhancing salt hypertension. The data available from several sources indicate that it is neither sweetness, as the term is commonly understood, nor the calorie value which endows certain sugar solutions with the appeal they have for rats.

The authors are indebted to Dr. John E. Overall for his invaluable aid in performing the statistical analyses.

1. Sapirstein, L. A., Brandt, W. L., Drury, D. K., Proc. Soc. Exp. Biol. and Med., 1950, v73, 82.

2. Koletsky, S., Lab. Invest., 1948, v7, 377.

3. Dahl, L. K., J. Exp. Med., 1961, v114, 231.

4. Hall, C. E., Hall, O., Lab. Invest., 1964, v13, 1471.

5. ———, Texas Rep. Biol. and Med., 1964, v22, 529.

6. ———, Lab. Invest., 1965, v14, 285.

7. ———, Texas Rep. Biol. and Med., in press.

8. Richter, C. P., Campbell, K. H., J. Nutrition, 1940, v20, 31.

9. Ostle, B., Statistics in Research, 2nd Ed., Iowa State Univ. Press, Ames, 1963, p132.

10. The Dispensatory of the United States of America, 25th Ed., J. Lippincott Co., Philadelphia and Montreal, 1955.

11. Hall, C. E., Hall, O., Fed. Proc., 1966, v25, 572.

12. Collier, G., J. Exp. Psychol., 1962, v64, 184.

13. Fisher, G. L., Pfaffman, C., Brown, E., Science, 1965, v150, 506.

14. Adolph, E. F., Am. J. Physiol., 1947, v151, 110.

Received December 16, 1965. P.S.E.B.M., 1966, v122.