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SEROSAL ACCUMULATION OF AMINO ACIDS AND HEPATIC LIPID LEVELS OF RATS FED SEVERAL CARBOHYDRATES*

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SUMMARY

The influence of the type of dietary carbohydrate on the *in vitro* serosal accumulation of amino acids in rat intestinal tissue was examined. In addition, the hepatic concentration of several classes of lipids was determined.

1. Food consumption and body weight gains of rats fed raw potato starch were significantly higher than those fed cooked potato starch, cornstarch, sucrose, glucose, or dextrin. Rats fed sucrose exhibited significantly lower body weight gains than those on other treatments.

2. The *in vitro* serosal accumulation of amino acids was lowest in rats fed raw potato starch and sucrose. These animals also exhibited the greatest and least weight of intestinal tissue per unit of length, respectively. There was little difference between other dietary treatments in terms of serosal accumulation rate, although the tissue of dextrin-fed rats tended to show a higher rate of passage.

3. Significantly higher levels of total lipid, free cholesterol, and neutral fat were found in the hepatic tissue of rats fed sucrose. There were no treatment differences in the levels of total cholesterol, phospholipids, free fatty acids, or the activity of glucose-6-phosphate dehydrogenase or malate dehydrogenase.

INTRODUCTION

Various carbohydrates are known to elicit different nutritional responses in experimental animals[1]. The type of dietary carbohydrate has been shown to affect protein utilization[2,3] and the levels of specific serum proteins[4]. Physiological changes attributed to specific carbohydrates, sucrose in particular, may partially be due to an alteration in the ability of intestinal tissue to transport one or more amino acids. It was previously proposed[5] that one of the effects of sucrose was to lower the availability of one or more essential amino acids. Individual hexoses have since been shown to affect the absorption of amino acids from the small intestine of the dog[6]. In addition, rats fed either fructose or galactose exhibited defects in the intestinal transport of certain amino acids[7].

Increased levels of total liver lipids, not noted in animals fed glucose or starch,

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have been shown to occur in rabbits fed sucrose as the dietary carbohydrate[8]. Changes in lipid composition were also noted and appeared to be dependent upon the type of carbohydrate and the level of consumption. The replacement of a portion of dietary glucose with sucrose has been reported to cause a progressive increase in serum cholesterol levels in the human[9]. Fructose caused elevated serum and liver triglyceride levels in the rat[10]. However, it was suggested that the observed changes may be influenced by animal age.

Thus, the physiological effects of carbohydrates, such as sucrose, are generally known. However, it is not clear whether a comparison of the type of starch or hexose fed would reveal an influence on the subsequent ability of intestinal tissue to transfer one or more amino acids.

The object of this study was to compare several different dietary carbohydrates with respect to their influence on the *in vitro* transfer of amino acids by intestinal tissue and on the levels of total liver lipid and its major constituent classes.

METHODS

Eighty male Sprague–Dawley rats, weighing 70–80 g, were divided into 6 groups with 8 rats per group. The rats were individually fed the experimental diets *ad libitum* for a period of 30–35 days. The dietary carbohydrates consisted of raw potato starch, cooked potato starch, corn starch, dextrin, sucrose, and glucose. The basal diet consisted of wheat gluten, 24 %; hydrogenated vegetable oil, 5 %; purified cellulose, 5 %; salt mixture (USP XIV), 4 %; vitamins in sucrose (Nutritional Biochemicals), 1 %; and carbohydrate, 61 %.

Food was removed 12 h prior to a transport experiment. One rat from each treatment, per day, was sacrificed by decapitation and a 5–6-cm segment of intestine was excised from a point 15 cm caudal to the pyloric sphincter. The segment was washed with Ringers phosphate buffer (pH 7.4)[11], weighed, and an everted Wilson–Wiseman sac containing 1 ml of phosphate buffer was prepared according to the technique described previously[12]. The preparation was placed in a 50-ml erlenmeyer flask in 10 ml of buffer containing L-amino acids (Mann Biochemicals) at concentrations based upon the approximate levels which would exist in a casein solution which is 9 mM with respect to lysine[13]. Several preparations from each treatment were incubated in buffer only to assess the presence of endogenous amino acids in the serosal fluid.

The flasks were gassed with oxygen for 30 sec, closed with a sleeve-type serum bottle stopper, and incubated under the conditions indicated. At the end of the incubation period, the serosal contents were collected and dried at 100° under a stream of dry N_2. We had previously determined[13] that none of the amino acids under consideration appeared to be affected by this procedure. Amino acids were determined by gas–liquid chromatography according to the procedure of Coulter and Hahn[14]. The transported acids were quantitated by comparison of peak height and retention time with average values obtained from repeated analysis of known quantitative standards at several different concentrations.

The livers were excised and total lipids were extracted and purified according to Folch *et al.*[15]. The yield of purified lipid was determined by weighing. Neutral lipid was estimated gravimetrically after stepwise elution of a portion of the total

lipid from a silicic acid column[16]. Recovery of the other lipid classes appeared to be incomplete with this procedure. Therefore, aliquots of the total lipid extract were analyzed for lipid phosphorus[17] and free and total cholesterol[18]. Free fatty acids were extracted from a light petroleum solution of the lipid extract with 0.05 M aqueous KOH. The alkaline extract was reextracted with light petroleum after acidification[19]. The quantities of free fatty acids present were determined by titration[20]. The activities of glucose-6-phosphate dehydrogenase[21] and malate dehydrogenase[22] were also determined.

Analysis of Variance and Duncan's multiple range test[23] were used to identify significant differences between all variables measured. During the analysis of known standards, lysine appeared to vary in regard to the formation of a quantitative derivative for gas–liquid chromatography. Therefore, the observed values for all amino acids were tested for homogeneity of variance[24] in order to insure that differences were due to treatment and not to variations occurring during the procedure used for derivative formation.

RESULTS

Effect of dietary carbohydrates on food consumption and body weight gain

Animal response to the dietary carbohydrates is shown in Table I. Rats receiving raw potato starch exhibited a markedly higher food consumption and gain in body weight than animals on other treatments. The food consumption of rats fed either cooked potato starch or sucrose was nearly identical, however, sucrose-fed rats gained significantly less body weight than either those fed cooked potato starch or cornstarch, dextrin and glucose. There was no difference in the food consumption of rats fed cornstarch, dextrin and glucose, however, glucose-fed rats exhibited a significantly higher gain in body weight compared to those fed cornstarch and dextrin.

TABLE I

EFFECT OF DIETARY CARBOHYDRATES ON FOOD CONSUMPTION AND BODY WEIGHT GAIN

The rats were individually fed for a period of 30 days prior to sacrifice. Values shown are the average for 8 animals per treatment and the standard error of the mean. Means sharing a common superscript are not significantly different ($P<0.05$).

Treatment	*Food consumption (g/day)*	*Weight gain (g/day)*
Raw potato starch	18.23 ± 0.75^{a}	3.51 ± 0.64^{a}
Cooked potato starch	9.39 ± 0.50^{b}	1.81 ± 0.39^{b}
Sucrose	9.12 ± 0.59^{b}	0.57 ± 0.06^{c}
Corn starch	11.13 ± 0.75^{c}	1.15 ± 0.10^{d}
Dextrin	11.33 ± 0.36^{c}	1.49 ± 0.08^{d}
Glucose	11.45 ± 0.54^{c}	1.91 ± 0.16^{b}

Effect of carbohydrates on the serosal accumulation of amino acids

The final concentrations of amino acids in the serosal medium is shown in Table II. Endogenous amino acids were not detected to any appreciable extent in

TABLE II

FINAL CONCENTRATIONS OF AMINO ACIDS IN SEROSAL FLUIDS

The intestinal sacs were incubated at 37° for 1 h at 90 oscillations/min. The incubation medium contained L-amino acids in the concentrations indicated in the table. Glucose was present at 27.7 mM concentration. The serosal fluid was collected and analyzed for amino acids. Quantities of serosal amino acids present were corrected for volume changes in the amount of fluid and calculated on a mM concentration basis. Values shown are averages and the standard error of the mean. Means within each column bearing a common superscript are not significantly different ($P<0.05$).

Treatment	*Val*	*Ile*	*Leu*	*Thr*	*Met*	*Phe*
	Initial concentration in mucosal medium (mM)					
	6.60	10.27	15.75	4.24	4.25	4.00
	Final serosal concentration (mM)					
Raw potato starch	5.82 ± 0.62^{a}	7.04 ± 0.56	6.20 ± 0.44	5.66 ± 0.47	6.83 ± 0.11	$5.56 \pm 0.46^{a,b}$
Cooked potato starch	8.22 ± 0.56^{a}	8.35 ± 0.50	8.39 ± 0.96	6.95 ± 0.68	8.11 ± 0.23	$7.60 \pm 0.51^{a,b}$
Corn starch	8.12 ± 0.59^{a}	7.62 ± 0.50	8.95 ± 1.18	6.05 ± 0.46	8.94 ± 0.67	$7.74 \pm 0.75^{a,b}$
Sucrose	4.13 ± 0.36^{b}	6.12 ± 0.61	6.00 ± 0.49	4.86 ± 0.30	5.12 ± 0.21	4.73 ± 0.50^{a}
Glucose	8.42 ± 0.66^{a}	9.09 ± 0.54	9.40 ± 0.67	6.78 ± 0.41	7.54 ± 0.20	$7.50 \pm 0.40^{a,b}$
Dextrin	8.17 ± 0.55^{a}	9.70 ± 0.50	9.36 ± 0.67	7.94 ± 0.77	8.35 ± 0.21	8.82 ± 0.40^{b}

TABLE III

EFFECT OF DIETARY CARBOHYDRATES ON INTESTINAL TISSUE THICKNESS AND THE SEROSAL ACCUMULATION OF AMINO ACIDS

The intestinal sacs were incubated under the conditions indicated in Table II. Serosal accumulation values were calculated on the basis of μM of each amino acid in the serosal medium and fresh tissue weight. The sac weights for each treatment represent an average weight of 8 sacs measuring 5 cm in length. Values shown are the average of 8 animals per treatment and the standard error of the mean. Means within each column sharing a common superscript are not significantly different ($P<0.05$).

Treatment	*Sac wt.* (*mg*)	(*μmoles amino acid per g of fresh tissue per h*)					
		Val	*Ile*	*Leu*	*Thr*	*Met*	*Phe*
Raw potato starch	481 ± 26^{a}	10.63 ± 1.6^{a}	14.23 ± 1.4^{a}	13.34 ± 1.4^{a}	10.42 ± 1.4^{a}	12.37 ± 1.2^{a}	10.91 ± 1.8^{a}
Cooked potato starch	415 ± 11^{b}	17.87 ± 1.5^{b}	18.11 ± 1.3^{b}	22.32 ± 2.8^{b}	15.16 ± 1.9^{b}	17.66 ± 1.8^{b}	$17.78 \pm 1.9^{b,c}$
Corn starch	376 ± 17^{b}	16.65 ± 1.3^{b}	18.64 ± 1.2^{b}	22.71 ± 1.9^{b}	17.07 ± 2.4^{b}	24.08 ± 1.9^{c}	14.81 ± 2.3^{b}
Sucrose	333 ± 23^{c}	11.09 ± 1.0^{a}	13.12 ± 1.2^{a}	12.82 ± 1.4^{a}	12.31 ± 1.5^{a}	12.36 ± 1.46^{a}	12.11 ± 1.4^{a}
Glucose	385 ± 10^{b}	18.97 ± 1.6^{b}	20.44 ± 1.2^{b}	21.11 ± 1.5^{b}	15.29 ± 1.1^{b}	16.96 ± 1.3^{b}	$16.89 \pm 1.0^{b,c}$
Dextrin	411 ± 25^{b}	21.14 ± 1.9^{b}	25.72 ± 2.4^{d}	25.86 ± 1.9^{b}	20.55 ± 2.6^{c}	21.04 ± 1.6^{c}	22.48 ± 2.0^{c}

TABLE IV

EFFECT OF DIETARY CARBOHYDRATES ON HEPATIC LIPID COMPOSITION

Values shown are the average of 8 animals per treatment and the standard error of the mean. Means sharing a common superscript are not significantly different ($P<0.05$).

Treatment	*Total lipid* (*mg/g liver*)	*Phospholipids* (*mg/g lipid*)	*Cholesterol* (*mg/g lipid*)		*Neutral fat* (*mg/g lipid*)	*Free fatty acids* (*μequiv/g liver*)
			Total	*Free*		
Raw potato starch	37.65 ± 1.38^{a}	19.43 ± 0.47^{a}	3.57 ± 0.14^{a}	2.18 ± 0.16^{a}	13.32 ± 0.17^{a}	2.37 ± 0.09^{a}
Cooked potato starch	43.29 ± 1.35^{a}	18.48 ± 0.98^{a}	3.70 ± 0.49^{a}	2.80 ± 0.30^{a}	17.66 ± 0.43^{a}	2.42 ± 0.20^{a}
Corn starch	41.01 ± 1.72^{a}	21.73 ± 0.49^{a}	4.01 ± 0.31^{a}	2.61 ± 0.19^{a}	13.61 ± 0.29^{a}	2.71 ± 0.14^{a}
Sucrose	56.23 ± 1.42^{b}	18.51 ± 1.09^{a}	4.83 ± 0.49^{a}	3.85 ± 0.17^{b}	31.35 ± 0.54^{b}	2.71 ± 0.11^{a}
Glucose	45.24 ± 2.68^{a}	18.70 ± 0.73^{a}	4.43 ± 0.08^{a}	2.89 ± 0.25^{a}	20.03 ± 0.54^{a}	3.33 ± 0.11^{a}
Dextrin	39.37 ± 1.93^{a}	17.05 ± 0.94^{a}	3.85 ± 0.25^{a}	2.53 ± 0.17^{a}	15.32 ± 0.31^{a}	3.36 ± 0.21^{a}

control preparations. Results for lysine are not shown due to a variable extent of derivative formation for gas–liquid chromatography. All other amino acids underwent uniform and complete derivatization In all cases, the lowest amino acid concentrations were noted in the serosal fluid of rats receiving either raw potato starch or sucrose as the dietary carbohydrate. Significant differences ($P < 0.05$) were found for only valine and phenylalanine, however, a general pattern of concentration differences is evident. The differences were more pronounced when the rate of serosal accumulation, based on the amount of intestinal tissue, were calculated (Table III). The average weight of intestinal segments of similar length was significantly ($P < 0.05$) greater for rats fed raw potato starch and significantly less from animals which had received sucrose as the dietary carbohydrate.

TABLE V

EFFECT OF DIETARY CARBOHYDRATES ON HEPATIC GLUCOSE-6-PHOSPHATE AND MALATE DEHYDROGENASE

Values shown are average of 8 animals per treatment and the standard error of the mean.

Treatment	*Glucose-6-phosphate dehydrogenase (units/g liver)*	*Malate dehydrogenase (units/mg liver)*
Raw potato starch	2.38 ± 0.18	2.22 ± 0.16
Cooked potato starch	2.01 ± 0.20	1.89 ± 0.16
Cornstarch	1.57 ± 0.11	1.79 ± 0.11
Sucrose	2.45 ± 0.16	2.01 ± 0.09
Glucose	1.52 ± 0.15	1.73 ± 0.10
Dextrin	1.68 ± 0.09	1.90 ± 0.06

The rate of serosal accumulation, for all amino acids, was significantly lower in tissue from rats fed either sucrose or raw potato starch. The accumulation rate was, in general, similar for rats which had received the other dietary carbohydrates. The rate tended to be highest in rats fed dextrin and was significantly higher in the case of isoleucine, threonine, and phenylalanine. Methionine transfer was also high in the tissue of rats fed cornstarch while none of the other amino acids appeared to show this trend.

Effect of carbohydrates on the concentration of hepatic lipids

The amounts of hepatic lipid classes determined are shown in Table IV. The highest amounts of total lipid, free cholesterol, and neutral fat were found in liver tissue from rats fed sucrose. No significant differences were apparent in the concentration of phospholipids, total cholesterol, or free fatty acids.

Effect of carbohydrates on the activity of liver enzymes

The activities of hepatic glucose-6-phosphate dehydrogenase and malate dehydrogenase are shown in Table V. No significant differences in the level of activity of either enzyme was apparent between the carbohydrate treatments.

DISCUSSION

It has been reported[8] that rabbits fed sucrose, particularly as the carbohydrate component of low-protein diets, suffered a severe loss in body weight and increased liver lipid. A similar response was noted in the case of rats fed sucrose in the present study. The markedly low gain in body weight of the animals appeared to be out of proportion to food intake when compared to the response of rats fed cooked potato starch since these animals gained considerably more weight with a similar food intake. In comparing these results with the findings of other investigators[2,3], it appears that the inclusion of sucrose as the dietary carbohydrate accentuated a caloric imbalance which was associated with a lower body weight gain and an increase in liver lipid.

Raw potato starch has been previously shown to be poorly utilized and causes a lowered protein utilization[2]. The daily food intake of the animals, however, was so high that it had the effect of overcoming lowered protein utilization and causing a significantly higher gain in body weight. The cooking of potato starch has been found to improve protein utilization. However, under the present conditions, the final product was of a rather hard, gritty consistency which may have contributed to the low food intake of the animals receiving the particular diet. The gains, however, were higher than that of rats fed cornstarch or dextrin. The low gains of rats fed sucrose thus appeared to be the result of one or more metabolic alterations caused by the carbohydrate.

The advantages and disadvantages of the everted sac technique for studying intestinal transport have been discussed[25]. The method yields no information concerning actual carrier mechanisms involved since the results indicate only the translocation of material across the intestinal epithelium. Nevertheless, a number of studies utilizing the method have appeared[26,27] and, if standard conditions and controls are used, one can assess the effects of various treatment on intestinal transport of various substances.

In the present study, there appeared, in most cases, to be little relationship between the rate of serosal accumulation of amino acids *in vitro* and the animal response to dietary treatment. When the final serosal concentrations were calculated, no significant differences existed except in the case of valine and phenylalanine. These two amino acids were present in lower concentrations in the serosal fluid of preparations from rats fed either raw potato starch or sucrose. The same trend was apparent for the other amino acids but the differences were not significant. When the rate of transfer was calculated on the basis of tissue weight, however, the lowest rate of transfer occurred in tissue preparations of the higest and lowest weights. Since the actual amount of each amino acid in the serosal fluid tended to be somewhat low in preparations from rats fed raw potato starch, the heavier tissue weight caused significantly lower values in terms of transfer rate. Conversely, the low transfer rate in the case of rats fed sucrose was the result of both low tissue weight and the small actual amount of each amino acid which appeared in the final serosal medium.

The differences in the weight of the tissue preparations was a reflection of the thickness of the intestinal wall since all segments were of similar length. An alteration in water content was presumably the primary cause. Unfortunately, this parameter was not measured. In animals fed raw potato starch, the rate of serosal accumulation appeared to be slower because of the increased thickness of the intestinal epithelium

and, possibly, an alteration in osmotic pressure relationships. It is possible that the slower transfer rate may be related to the lowered protein utilization caused by the starch, however, it would be difficult to relate the lowered transfer rates to the high food consumption and rate of gain.

A relationship between serosal amino acid accumulation and animal response may exist in the case of rats fed sucrose since the low transfer rate for all amino acids was in accord with the low weight gains. The rate of serosal accumulation, in this case, could not be explained on the basis of the thickness of the intestinal epithelium since the slow transfer rate occurred in much thinner preparations. Thus, the proposed lower availability of certain amino acids in sucrose-fed rats[5] was possibly related to a lowered rate of intestinal transfer of essential amino acids. The overall results, however, appear to support the conclusion[28] that carbohydrates do not inhibit amino acid transport mechanisms and the observed differences are more probably the result of altered osmotic relationships.

An increase in nearly all classes of hepatic lipid has been reported in rabbits fed sucrose[8]. While we did not find any differences in total phospholipids or cholesterol, it has recently been pointed out that not all strains of rats respond to dietary sucrose in the same manner[29]. The response to dietary carbohydrates may also be influenced by age[10] and species of animal. Under our conditions, the increase in total lipid consisted largely of an accumulation of the neutral fraction. These results are similar to those recently noted in comparing the effects of sucrose and dextrin in choline-deficient rats[30]. Thus, although sucrose may exert a deleterious effect upon intestinal amino acid transfer, it appears that the more significant effect on growth is caused by the development of a metabolic pattern which causes an excessive rate of hepatic lipid synthesis. An excessive activity of glucose-6-phosphate dehydrogenase in the liver tissue of sucrose-fed rats has been implicated in the higher levels of lipid which occur. We did not note significant differences between treatments in regard to the levels of glucose-6-phosphate dehydrogenase, although there was a slight trend toward higher levels of activity in rats fed sucrose. Neither of the patterns noted in activity of the two enzymes appeared to bear much relationship to the levels of lipid classes observed. Although hepatic enzymes may be influenced by the type or amount of dietary carbohydrate to alter lipid levels, there are other undetermined factors which are possibly involved. In addition, the influence of rat strain and age may have a bearing upon the relationship of hepatic enzyme activity to the observed changes in the level of liver lipid and its constituent classes.

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