Comparative Effects of Dietary Sucrose and Cornstarch on Tryptophan Requirement of the Rat.* (22030)

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Available data indicate that the growth increment of rats fed low protein rations is dependent to a considerable degree on the source of dietary carbohydrate. Krehl *et al.*(1), Henderson *et al.*(2), Harper and Katayama (3) and others(4-6) observed that dextrin or cornstarch support better growth of rats fed low casein rations than does sucrose. Similar results have been reported by Womack *et al.* (7) for rats fed a low level of an amino acid mixture. However, the differences in growth on these diets were eliminated when the casein content of rations or the level of the amino acid mixture was increased. In the present communication data are presented indicating that the source of dietary carbohydrate may also affect growth increment of rats fed a diet deficient in a single amino acid.

Procedure. Two basal rations were employed, diets A and B. Diet A was a tryptophan-free ration of the following composition in percent: sucrose, 60.8; acid hydrolyzed casein,† 24; cottonseed oil (Wesson), 10; salt mixture,‡ 5; and l-cystine, 0.2. To each kg were added the following crystalline vitamins: thiamine hydrochloride, 40 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 100 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; vit. B_{12}, 150 μg; 2-methyl-naphthoquinone, 5 mg; choline chloride, 2 g; vit. A, 5000 U.S.P. units; vit. D_2, 500 U.S.P. units; and alpha tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. Diet B was similar in composition but contained cornstarch in place of sucrose as the dietary carbohydrate. In addition to the basal rations the following diets were also employed, consisting of basal rations A and B plus each of the following supplements: (a) 600 mg dl-tryptophan/kg diet (b) 1.8 g dl-tryptophan/kg diet and (c) 5.4 g dl-tryptophan/kg diet. These supplements were incorporated in the diet in place of an equal amount of hydrolysate and corresponded to 0.25%, 0.75% and 2.25% respectively of the hydrolysate content of the basal ration. In addition to above diets, experiments were also conducted with diets which were identical to basal rations A and B except that the acid hydrolysate was replaced by intact casein.§ Two hundred albino female rats of the University of S. California strain, were selected at 21 to 24 days of age and average body weight of 42 g (range 36 to 50 g). Animals were placed in metal cages with raised screen bottoms and fed above diets *ad lib.* (20 rats/group). Diets were prepared weekly and stored under refrigeration. Rats were fed daily, and all food not consumed 24 hours after feeding was discarded. These measures were employed to minimize oxidative changes in the diet. Feeding was continued for 18 weeks or until death, whichever occurred sooner. Of the surviving animals, 12 in each group were autopsied and gonadal and uterine weights determined. Ovaries were fixed in Bouin's solution, and sections were prepared and stained with hematoxylin and eosin.

Results are summarized in Table I. Findings indicate that the weight increment of immature rats fed a tryptophan-free diet supplemented with 600 mg dl-tryptophan per kg diet was more than twice as great on

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‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, O.

TABLE I. Effects of Dietary Sucrose and Cornstarch on Body, Ovarian and Uterine Weights of Rats Fed a Tryptophan Deficient Ration Supplemented with Graded Levels of dl-tryptophan (20 Animals per Group).

Dietary group	Tryptophan supplement, g/kg of diet	Increment in body wt after 18 wk,* g	Ovarian wt, mg	Uterine wt, mg
S†	.6	57 ± 5.1	18	66
C	.6	128 ± 5.2	45	348
S	1.8	152 ± 4.1	42	300
C	1.8	148 ± 6.0	51	380
S	5.4	146 ± 5.3	49	263
C	5.4	151 ± 3.7	47	298
S	Intact casein	199 ± 4.9	58	398
C	" "	200 ± 6.0	64	483

* Including stand. error of the mean.
† S = Sucrose; C = Cornstarch.

the cornstarch-containing ration than on diet containing sucrose as the source of carbohydrate. Average weight increment of rats at this level of tryptophan supplementation after 18 weeks of feeding was 128 ± 5.2‖ g in the corn starch series and 57 ± 5.1 g for animals fed the sucrose-containing diet. At higher levels of tryptophan supplementation (1.8 g and 5.4 g dl-tryptophan/kg diet), no significant difference in weight increment was observed between rats on cornstarch or sucrose-containing rations. Similarly, no significant difference in weight increment occurred between these two dietary groups when intact casein was fed in place of the hydrolysate. Animals fed unsupplemented tryptophan-free rations A and B lost weight from the start of feeding and died within 6 weeks of start of experiment. A marked difference was noted, however, between rats in the two groups. On the sucrose-containing diet animals developed an unthrifty appearance with ruffled fur and varying degrees of alopecia. On the cornstarch-containing ration, although weight loss of rats was the same as in the sucrose group, the fur remained smooth and sleek. After 28 days of feeding only 1 out of 20 rats fed the basal ration A was still alive in contrast to 16 out of 20 rats on ration B. The average survival time for rats on tryptophan-free diets was 24 ± 0.6 days (range 20 to 29 days) on ration A and 33 ± 1.3 days (range 23 to 41 days) on ration B.

At autopsy significant differences in ovarian and uterine weights were noted between cornstarch and sucrose series in rats fed a tryptophan-free ration supplemented with 600 ml dl-tryptophan/kg diet. On the sucrose-containing diet the ovaries and uteri appeared immature both in weight and microscopic appearance. These findings might have been anticipated since the corresponding organs of rats of similar weight which had been fed a natural food stock ration were also immature. In contrast rats in the cornstarch series at the same level of tryptophan supplementation had well developed ovaries and uteri¶ in addition to increased body weight. At higher levels of tryptophan supplementation (1.8 g and 5.4 g dl-tryptophan/kg diet) and on intact casein rations, the ovaries and uteri were well developed in both the sucrose and cornstarch-containing series.

The weight increment of rats fed dl-tryptophan at levels of 1.8 g and 5.4 g per kg of diet was significantly greater both in the cornstarch and sucrose series than that of animals receiving the lower level of supplement (600 mg dl-tryptophan per kg of diet) but was still approximately one-third lower than that of rats fed the intact casein. Since the 1.8 g level of supplementation was as effective as the higher level of supplement in promoting the weight increment of rats fed the hydrolysate-containing rations, and since the weight increment of rats fed these supplements both in the cornstarch and sucrose series was significantly less than that of animals fed the intact casein, it would appear that the acid hydrolysate employed in the present experiment may have been deficient not only in tryptophan but in some other factor or factors present in the intact casein.

‖ Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

¶ Subsequent data indicate that ovaries of rats in this group were normal functionally as well as in histologic appearance. After the 18th week of feeding, 6 animals in this series were bred to males of proven fertility. All 6 gave birth to living young which appeared normal in all respects except for size (young averaged 3.8 g at time of birth).

Discussion. Findings suggest that the source of dietary carbohydrate may profoundly affect the biological value of a protein. Rats fed a tryptophan deficient ration supplemented with a suboptimal level of dl-tryptophan (600 mg/kg of diet) grew poorly when sucrose was the dietary carbohydrate but showed a significant increment in body weight over that observed on the sucrose-containing diet when cornstarch replaced sucrose as the source of dietary carbohydrate. At higher levels of tryptophan supplementation (1.8 g or 5.4 g dl-tryptophan/kg of diet) no significant difference in weight increment occurred between rats fed cornstarch or sucrose-containing rations. Similar findings were obtained in respect to ovarian and uterine weights. Ovaries and uteri appeared infantile in rats fed a tryptophan-deficient ration supplemented with suboptimal levels of dl-tryptophan when sucrose was the dietary carbohydrate but appeared normal in size and appearance either when cornstarch replaced sucrose as the source of dietary carbohydrate or when the tryptophan content of the sucrose-containing ration was increased.

No data are available to account for the beneficial effects of cornstarch over sucrose for rats fed suboptimal amounts of tryptophan under conditions of the present experiment.** It has been suggested that essential amino acids may be synthesized by the intestinal flora of rats fed cornstarch(4) and it is possible that sufficient amounts of tryptophan may be available from this source to account for the observed results. The converse explanation—that the intestinal flora of rats fed sucrose use greater quantities of tryptophan than the intestinal flora of animals fed cornstarch—would appear, however, to be equally tenable(3). Another hypothesis is that the rate of digestion may be slower when rations containing cornstarch in place of sucrose are fed and that this may permit a more efficient utilization of tryptophan(5). Regardless of the final explanation of the observations reported above, however, findings clearly indicate that the source of dietary carbohydrate may, at least under certain experimental conditions, significantly modify the tryptophan requirement of the rat.

Summary. Immature female rats were fed tryptophan-free rations supplemented with graded levels of dl-tryptophan and containing either sucrose or cornstarch as the source of dietary carbohydrate. Rats fed dl-tryptophan at a level of 600 mg/kg of diet grew poorly when sucrose was the dietary carbohydrate but showed a significant increment in body weight over that observed on the sucrose-containing diet when cornstarch replaced sucrose as the source of dietary carbohydrate. At higher levels of tryptophan supplementation (1.8 g or 5.4 g dl-tryptophan/kg of diet) no significant difference in weight increment occurred between rats fed cornstarch or sucrose-containing rations.

** In view of the fact that some starches contain small amounts of protein, the possibility was considered that sufficient amounts of tryptophan were derived from this source to account for the observed results. Nessler nitrogen determinations of the cornstarch employed in this experiment, however, revealed no detectable N.

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