of hydrodextran following intravenous administration, three 1.75-kg male rabbits were given 30 ml each of the 6% solution. The hydrodextran content of the plasma was determined by colorimetric assay of the alcohol precipitate, after digestion of the plasma sample with potassium hydroxide(1).

Excretion study in man. Hydrodextran in 6% solution was administered intravenously to 2 subjects. Samples were taken to determine the plasma hydrodextran concentration. The total urine was collected and analyzed to determine the cumulative excretion of hydrodextran. The samples were analyzed by the alcohol precipitation method referred to(1). The amount given was 500 ml of a 6% solution, or 30 g of hydrodextran, injected over a 30-minute period. The picture observed was essentially the same as that reported for dextran itself(1).

Summary. Hydrodextran was prepared by high-pressure catalytic hydrogenation of dextran. The 6% solution prepared for intravenous use was found to be essentially stable over a 6-month period of storage. The preliminary studies on blood level and urine excretion showed no specific effect from hydrogenation of the terminal groups of the dextran chain.

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Growth Promotion in Rats by Crude Concentrates of the Bifidus Factor.* (20679)

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A growth-factor essential for a distinct strain of *L. bifidus* var. Penn is present in human milk and in particular in human colostrum. In contrast, the activity of cow's milk with regard to the same microbiological growth-factor is very low, on the average only 1/40th that contained in human milk(1-3). This "Bifidus Factor" occurs in low and high molecular form and appears to be related chemically to the blood group polysaccharides (1-3). Hog mucin and meconium, as well as other sources of blood group polysaccharide, have shown high microbiological activity for *L. bifidus* var. Penn(1-3). The recognition of a specific, microbiologically active factor in human milk made it desirable to investigate its possible role in the nutrition of animals and men. In the present study, supplements of crude sources of the Bifidus Factor were added to special rations fed young rats and their effect on growth and utilization of food was observed.

Material and methods. In the first and largest group of experiments a commercial infant formula was used,‡ which in its composition, at least in its content of total protein, fat and lactose, closely approximates that of human milk. The addition of concentrates of the Bifidus Factor should make this mixture even more similar to human milk. This dilute cow's milk formula was fed to young weanling rats of the Sprague Dawley strain *ad libitum*. Quantity consumed and weight of rats was measured daily. Control animals received the unsupplemented formula. The various supplements used in the experiments were as follows:

Exp. 1. Duration 8 weeks a) Charcoal eluate from human milk(4) with average microbiological activity of 300 γ per unit, 400 mg

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‡ S. M. A. liquid, Wyeth Laboratories. For use, contents of one can (13 oz.) was diluted with water to make 1 liter.

per 100 ml basal diet, b) Meconium-suspension (20%): 15 ml, equivalent to about 1000 microbiological units of Bifidus Factor, in 100 ml of basal diet, c) Hog mucin, granular (Wilson), microbiological activity 300 γ per unit, daily dose 50 mg per rat. *Exp. 2.* Duration 10 weeks a) Hog mucin, 600 mg per 100 ml of the basal diet, b) Human colostrum, with average microbiological activity 5 times higher than late human milk(2,3): 20 ml in 100 ml basal diet, c) Meconium-suspension (20%): 15 ml in 100 ml basal diet, d) "Activated" chitin(5). Chitin, prepared from crabshells, was treated with HCl and methanol(5), microbiological activity about 300 γ per unit, given in amount of 300 mg per 100 ml basal diet. *Exp. 3.* Duration 4 weeks a) Hog mucin, 400 mg per 100 ml of the basal diet, b) Hog mucin, hydrolyzed with HCl, microbiologically inactivated,§ given in equivalent amounts as unhydrolyzed hog mucin, c) Hog mucin treated with an enzyme preparation, obtained from *L. bifidus* var. Penn(1-3), microbiological activity reduced to about 20-30% of the original activity, given in equivalent doses as untreated mucin, d) Hog mucin mixed with inactive (boiled) "Bifidus enzyme", in the same amount as used for inactivation of hog mucin with active enzyme. In these last 2 preparations, the lyophilized enzyme preparation containing about 25% protein was added to an equal amount of hog mucin. Measured weight gains were compared with total protein intake over the entire period of the experiments, and the data were calculated according to customary statistical methods. The *basal diet* was considered to contain 70 cal/100 ml and 1.5 g protein/100 ml. 20% human colostrum in the basal diet should give values of 68 cal/100 ml and 1.8 g protein/100 ml(6). The milk eluate was free from protein. Mucin contained about 50% of protein. No similar calculations were required for meconium and "activated" chitin, which were found to have no growth promoting effect. In Exp. 4, the same dilute milk-formula was used as in Exp. 1-3 for a period of 8 weeks. The formula was supplemented with most of the known vitamins, *i.e.*, A, D, E, K and those of

§ Kindly supplied by Dr. R. M. Tomarelli, Wyeth Laboratories, Mason, Mich.

TABLE I. Effect of Bifidus Factor on Growth and Food Efficiency in Rats Fed Cow's Milk Formula.

Exp.	Wk		Animals	Wt gain, g	Prot. intake, g	Cal. intake (hundreds)	Gain/Prot.	P*	Gain/Cal. (100)	P
I	8	Control	10	28.2 ± 2.5†	24.0 ± .3	11.2 ± .1	1.2 ± .1		2.6 ± .2	
		Milk eluate	8	42.8 ± 2.0	24.0 ± .3	11.2 ± .1	1.8 ± .1	.001	3.9 ± .2	.005
		Meconium	9	26.7 ± 1.5	23.0 ± .3	10.7 ± .1	1.2 ± .1		2.6 ± .2	
		Hog mucin, 50 mg/day	9	38.0 ± 2.3	25.0 ± .2	11.7 ± .1	1.6 ± .1	.001	3.4 ± .2	.005
II	10	Control	9	44 ± 2.8	31 ± .7	14.4 ± .32	1.4 ± .04		3.1 ± .09	
		Hog mucin, 600 mg/100 ml	12	82 ± 3.4	48 ± .8	19.2 ± .32	1.7 ± .09	.01	4.3 ± .23	.001
		Colostrum, 20%	11	83.8 ± 3.5	48 ± .9	18.1 ± .36	1.7 ± .09	.01	4.6 ± .25	.001
		Chitin ("activated"), 300 mg/100 ml	12	48 ± 2.7	32 ± .8	14.9 ± .32	1.5 ± .06	.3	3.2 ± .13	
		Meconium, 15 ml 20%/100 ml	12	39.8 ± 3.2	32 ± .8	14.9 ± .32	1.2 ± .09		2.7 ± .20	
III	10	Control	10	16.4 ± 3.0	13.5 ± 1.0	6.3 ± .5	1.2 ± .12		2.5 ± .3	
		Hog mucin, .4%	10	42.0 ± 3.7	21.8 ± 1.7	9.2 ± .4	1.9 ± .10	.001	4.5 ± .2	.001
		Hydrol. hog mucin, .4%	10	17.0 ± 2.1	15.4 ± .9	6.5 ± .4	1.1 ± .12		2.5 ± .3	
		Mucin + enzyme, .4%	10	38.2 ± 3.8	21.8 ± 1.2	8.7 ± .5	1.8 ± .09	.001	4.3 ± .2	.001
		Mucin + boiled enzyme, .4%	9	33.0 ± 4.7	21.0 ± 1.5	8.4 ± .6	1.6 ± .15	.05	3.8 ± .3	.01

* P = Probability calculated from distribution of *t*, Fisher, R. A., and Yates, F., Statistical Tables, London, 1938, p. 26.

† All values following ± are stand. error of the mean: $\frac{\text{Stand. dev.}}{\sqrt{\text{No. observations}}}$.

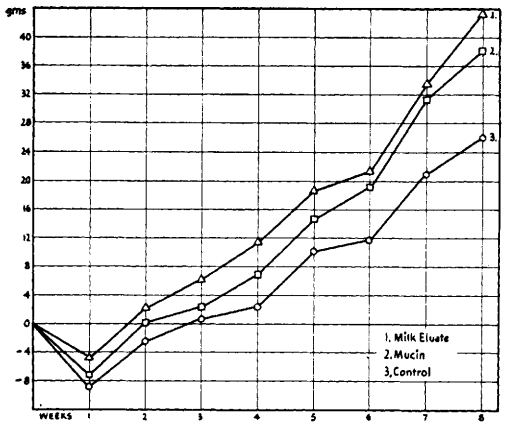

FIG. 1. Weight curves of rats receiving milk eluate and mucin compared with controls.

the vit. B Complex, including folic acid (10 γ daily), biotin (1 γ) and B_{12} (0.1 γ). The control group received 0.4% glucose added to the milk dilution, 4 experimental groups were supplemented with 0.4% of hog mucin, glucuronolactone,‖ galacturonic acid and pectin respectively.

In a second series of experiments, the basal diet consisted of 30% alcohol-extracted peanut meal, 6% vitamin-free casein, 40% sucrose, 20% lard and 4% salt mixture, with the usual vitamin supplements, except choline(7). This and similar rations were used for the production of fatty liver and hepatic cirrhosis. In this paper, only the growth-promoting effect of hog mucin is reported (Exp. 5). The results obtained in this experiment were confirmed in other similar experiments.

Results. The experimental observations (Exp. 1-3) made on rats fed dilutions of cow's milk as basal diet with or without supplement are summarized in Table I. The weight curves from Exp. 1 are charted in Fig. 1. The addition of mucin, of charcoal eluates prepared from human milk and of human colostrum have proved to have growth promoting effect. They also improved the utilization of ingested calories and of protein consumed, and thus of food efficiency. In contrast, meconium and "activated chitin", consisting chiefly of α- and β-methylglucosaminide(5) were found to be inactive. Acid hydrolysis of mucin deprived it of its growth promoting activity, whereas enzymatic inactivation up to 70-80% left the nutritional benefit exerted by mucin unimpaired.

The findings in Exp. 4 may be summarized as follows: *Exp. 4.* Average starting weight of rats in all 5 sub-groups 54.0 g. Weight-gain after 8 weeks in the experiment with glucose 41.5 $\pm$ 2.0, mucin 67.2 $\pm$ 3.3, glucuronalactone 41.8 $\pm$ 2.4, galacturonic acid 47.8 $\pm$ 3.1 and pectin 47.3 $\pm$ 3.1. The greater increase in weight in the group receiving mucin compared with all other control groups is highly significant ($P < 0.001$).

Even with the use of a high-protein alipotropic diet, consisting chiefly of peanut-meal, the growth promoting effect of hog mucin was clearly demonstrable over a prolonged experimental period (Table II).

Discussion. Human colostrum, eluates from human milk, and hog mucin, which all promote growth in rats under special experimental conditions, are characterized by high microbiological growth activity for *L. bifidus* var. Penn. On the other hand, "activated chitin" and meconium, both microbiologically active, were in comparable doses ineffective in animals. There are different possible explanations for this discrepancy:

1. Microbiological activity may be less specific and could be inherent to a varying number of chemically similar compounds, acting perhaps as precursors for the real growth-factor. In contrast, growth effect in animals is exerted only by compounds containing the "true" Bifidus Factor. Thus, meconium and "activated chitin" would not belong in this category.

2. Only high molecular forms of the Bifidus Factor(1-3) may influence, beneficially, growth in animals, probably through the intermediary of the intestinal flora. "Activated chitin" consists chiefly of low molecular compounds. Meconium may be ineffective, because its high molecular, microbiologically active constituents are unavailable to the intestinal flora of rats.

The growth effect in rats by colostrum, eluates from human milk, and hog mucin may not be due to the Bifidus Factor but to other, unidentified "contaminants". The fact that acid-hydrolyzed mucin was inactive would

‖ Kindly furnished by Corn Products, Inc.

TABLE II. Effect of Hog Mucin on Weight-Gain in Rats Fed an Alipotropic Peanut Meal Ration for 200 Days.

Exp.	Days		Animals	Starting wt, g	Final wt, g	P	Food intake ag. daily, g
V	200	Control	10	115.0 ± 3.0	292 ± 12.5		10.6 ± 0.3
		Hog mucin, 25 mg/day	10	115.7 ± 1.9	323 ± 6.0	.05	10.7 ± 0.4
		50 mg/day	9	115.4 ± 1.8	330 ± 8.1	.02	10.7 ± 0.4

rather support the determining role of the Bifidus Factor. On the other hand, the unimpaired activity of enzymatically treated hog mucin with greatly reduced microbiological activity does not favor, although it does not exclude this conclusion. It is conceivable that some special high molecular compounds of hog mucin, not inactivated by the enzymatic process, are the growth promoting agents for rats. Rearrangement through transglucosidation in the intestine is equally possible.

The results with hydrolyzed mucin, as well as those with small amount of mucin added to a high protein (peanut-meal) diet, and finally negative experiments(8) with substitution of mucin by equivalent quantity of protein (casein) seem to eliminate the possibility that the effect of crude mucin in animals may be due to its protein content. Furthermore, the active eluates obtained from human milk were free from protein and contained mainly bifidus-active polysaccharides.

The growth rate of the experimental animals, even when fed active concentrates, has been subnormal, due to the low total protein content of the basal dilute milk ration. Addition of vitamins, or of glucose, glucuronolactone, galacturonic acid, or pectin to the basal diet was without any effect on the growth rate, whereas hog mucin, polysaccharide fractions of human milk or colostrum have shown distinct growth promoting effect. Glucuronolactone(9-11) and other carbohydrates, such as chondroitin sulfuric acid(12-14) have been in the past credited to act under particular experimental conditions as growth promoting factors for chicks and rats. Chondroitinsulfuric acid is present in hog mucin(15). On the other hand, the fractions obtained from human milk are free from sulfur. Thus, at least for the fractions prepared from human milk, chondroitin sulfuric acid is not the growth promoting constituent.

Summary. Human colostrum, charcoal eluates prepared from human milk, and hog mucin, promoted growth in rats receiving a dilute cow's milk formula as basal diet. Supplements of known vitamins or of glucose, glucuronolactone, galacturonic acid, and pectin were ineffective. Rats receiving an alipotropic ration, containing peanut-meal and casein as source of protein, have shown better weight gain after supplementation with small daily doses of hog mucin than the unsupplemented controls. The question was raised and discussed whether these beneficial effects may be related to the Bifidus Factor activity of the supplements used.

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