

of "Tween 80" to the medium thus had no deleterious effect on motility and in fact may have enhanced it slightly. "Tween 80" (1:10) appeared to have no effect on motility of 12-hour cultures of *E. coli*, *S. paratyphi B*, *S. dysenteriae* and *Ser. marcescens*.

The influence of antiserum on motility was examined for 3 strains. Antisera prepared in rabbits for another purpose (Macdonald) (1), were used. Growth of each strain from a 3-day culture on blood agar was emulsified on a slide with homologous antiserum, covered with a coverslip and sealed with vaseline. Control emulsions in normal serum from the same rabbits were similarly prepared. All preparations were incubated at 37°C for 30 minutes and then examined by darkfield. The numbers of motile and non-motile cells in 10 fields were counted (a total of approximately 300 to 500 cells for each preparation).

In the control preparations motility was found in about 40% of cells of strain B8-3, about 5% of cells of strain WT-OZ, and about 2% of cells of strain MU-3. In emulsions prepared with antiserum, no motility was

found.

Summary. Progressive gliding motility, lashing around a fixed pole, and flexion were found in five strains of *Fusobacterium girans* when examined in fluid suspensions by dark-field microscopy. Electron, phase contrast, and polarized light microscopy failed to reveal flagella on these organisms. The presence of a slime layer was indicated by electron microscopy, and by the use of India ink preparations. Motility apparently was not affected by the addition to the medium of either "Tween 80" or alkylated aryl sulphonate. Motility was inhibited in the presence of specific antiserum. No explanation is offered for the mechanism of motility in *Fusobacterium girans*.

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Hydrodextran: Preparation, and Study on Blood Level and Excretion Rate. (20678)

PHILIP F. TRYON AND WALTER L. BLOOM. (Introduced by Arthur P. Richardson.)

From the Research and Development Laboratories, Commercial Solvents Corp., and the Departments of Biochemistry and Medicine, Emory University.

Native dextran is a long-chain glycosidic polymer of glucose produced by *Leuconostoc* fermentation of sucrose. For clinical use in blood-volume replacement, the native dextran is split by acid hydrolysis and then fractionated with methanol, to obtain shorter chain units in the range of 50-100,000 molecular weight. Each of these chains terminates in the hemiacetal group of the end-glucose unit. This reactive end-group is a possible source of destructive side reactions of dextran in solution, as pointed out by Zief and Stevens. These authors report the preparation of a hydrodextran in which the terminal hemiacetal groups are reduced to alcohol groups (4).

We have prepared a similar hydrodextran by means of high-pressure catalytic hydrogenation of clinical-fraction dextran. With the resultant modification in the terminal group of the polymer chain, it seemed possible that the mechanism of excretion from the blood stream might be different. For the blood level studies a 6% solution of hydrodextran, with 0.9% of sodium chloride, was prepared. In rabbits, and in man, it was

TABLE I.
Hydrogenation of Dextran, 12% Solution.

Analysis	Initial	Final
Viscosity, centistokes	6.65	6.22
Glucose equivalent, mg %	114	1.0

TABLE II. Stability of Hydrodextran, 6% Solution.

Specification	Initial	25°C	40°C	60°C
Hyrodextran, %	5.81	5.80	5.79	5.79
Molecular wt, wt avg	*	78000	81000	86000
Viscosity, centistokes	2.86	2.81	2.78	2.76
Glucose equiv., mg %	2.1	2.2	2.5	3.0
pH	6.7	6.5	6.5	6.5
Buffer capacity, ml 0.01N NaOH/l	0.5	0.8	0.8	0.7
Color, A.P.H.A.	15.	20.	20.	40.

* The molecular wt of the lower 5-10% of total material was 15000, that of upper 5-10% was 350000.

found that the blood level values progressively decreased in a pattern similar to that observed with dextran itself. No specific effect of the modified end-group was noted in these studies.

Preparation of hydrodextran. The initial dextran was prepared by acid hydrolysis of native dextran, followed by fractional precipitation with methanol to obtain material of 50-100,000 molecular weight. One hundred g of dextran was dissolved in 750 ml of water to give a 12% solution, and placed in an 1800-ml stainless steel rocking bomb together with 20 g of Raney nickel catalyst. The bomb was placed under 1000 lb pressure of hydrogen, and maintained at 140°C for 2 hours. The hydrogenated solution was filtered to remove the catalyst. As an indication of the course of reaction, the viscosity and glucose equivalent were run on both the initial and final solution. The glucose equivalent, as a measure of terminal glucose units, was determined by the Somogyi method(2). The hydrodextran was precipitated from the hydrogenated solution by the addition of 1.5 volumes of methanol. After standing overnight, the supernatant liquid was decanted and the solidified cake was dissolved by heating on the steam bath with 0.4 volume of water, based on the volume of hydrogenated solution. From this concentrate, the hydrodextran was reprecipitated as a fine, white powder by dropwise addition to 6 volumes of methanol, based on the volume used for the concentrate, with vigorous agitation. After partial settling, the supernatant liquid was decanted and replaced with fresh methanol. This slurry was filtered, the product was washed in the funnel with additional methanol, and air-dried. The final drying was for 20 hours in

a vacuum oven at 10 mm and 110°C. The yield was 90% on a weight basis. In a solution of 6 g per 100 ml, the viscosity was 2.76 centistokes, and the glucose equivalent was 0.5 mg %.

Preparation of hydrodextran, 6% sol. For studies on administration of hydrodextran, the material was made up as a 6% solution with 0.9% of sodium chloride. The solution was filtered through a bacterial filter, drawn into 500-ml plasma-type bottles, and autoclaved for one hour at 250°F for final sterilization. A series of specification tests was run on this 6% solution, using the methods described for analysis of 6% dextran solution(3). For a measure of stability, 3 of the bottles were stored at different temperatures, and the specification values were redetermined after a period of 6 months. The data indicate a relatively stable preparation.

Excretion study in rabbits. For a preliminary study of the duration of plasma levels

TABLE III.
Duration of Plasma Hydrodextran in Rabbits.

Time	Plasma level, mg %		
	1	2	3
Control	3.5	3.5	4.5
Immediate	1332	1672	1540
24 hr	285	339	346
48 "	84	115	76

TABLE IV.
Duration and Excretion of Hydrodextran in Man.

Time	Plasma level, mg %		Total in urine, g	
	1	2	1	2
Control	21	43	—	—
Immediate	723	723	—	—
6 hr	284	303	16	17
12 "	260	250	17	18
24 "	202	190	18	18
48 "	133	108	18	18

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)

PAUL GYÖRGY, MARIA I. MELLO,[†] F. E. TORRES, AND L. A. BARNES.

From the Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia.

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.