TABLE II.
Effect of Thiourea on Blood Sugar Level of 12 Non-fasted Rats Previously Fed KIO_3. Sugar reading (B) was taken 2½ hr after rats received 10 mg/kg thiourea intraperitoneally.

Sex	Wt g	Blood Sugar (A) mg/100 cc	Blood Sugar (B) mg/100 cc	Difference (B-A)
M	383	63	79	16
M	392	59	86	27
M	366	76	90	14
M	333	90	93	3
M	319	63	97	34
F	285	93	105	12
F	315	105	122	17
F	186	102	97	—5
F	147	69	86	17
M	193	83	97	14
M	201	90	97	7
M	150	73	93	20
			Avg Blood Sugar Incr.	14.7

of 5.16 indicating less than one chance in a thousand of the results being accidental.

Summary. Oral administration of potassium iodate for a 2-day period in drinking water prevented the hyperglycemia which followed thiourea injection in rats.

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Microbial Synthesis and Multiple Nature of *Lactobacillus bulgaricus* Factor. Possible Role in Chick Nutrition.* (17778)

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Recently Williams, Hoff-Jorgensen and Snell(1) reported that many natural products contained a previously unrecognized factor necessary for the growth of several strains of *Lactobacillus bulgaricus*. An assay method was described in which yeast extract was used as a standard of comparison and given an arbitrary potency of one unit per mg. Application of this assay method to culture filtrates of several microorganisms revealed that the *L. bulgaricus* factor (LBF) was generally synthesized and excreted in part into the medium by organisms which did not require it preformed (Table I). Such culture filtrates were in some cases appreciably more potent sources of LBF than is yeast extract. The richest source of LBF yet found is the culture filtrate from *Ashbya gossypii* NRRL—1056, which on the dry basis is 3 to 5 times as active as yeast extract. LBF is also produced by *A. gossypii*, although to a lesser extent, in a synthetic medium containing glucose, amino acids, salts and vitamins. Fractionation characteristics of LBF(1) suggested that more than a single active entity might occur naturally. To check this possibility, culture filtrates of *A. gossypii* were chromatographed on paper strips in a manner similar to that described by Winsten and Eigen(2). Water-saturated

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1. Williams, W. L., Hoff-Jorgensen, E., and Snell, E. E., *J. Biol. Chem.*, 1949, v177, 933.

2. Winsten, W. A., and Eigen, E., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 513.

TABLE I.
Synthesis of LBF by Microorganisms.

Organisms	Type of medium	Units of LBF in growth medium per ml	per mg solids
Candida crusoides (arborea)	Synthetic*	6	0.6
Mycotorula lipolytica	,,	7	0.7
Saccharomyces cerevisiae Y-30	,,	6	0.6
Torula utilis	,,	6	0.6
Aspergillus niger	,,	12	0.2
Penicillium chrysogenum 1380	,,	15	1.7
,, ,,	Corn-steep, glucose†	42–72	1.5–2.7
Ashbya gossypii NRRL—1056	Grain stillage, animal stick liquor, glucose†	75–125	3–5
Lactobacillus pentoaceticus	Yeast water, glucose†	24	1.0

* The synthetic medium varied slightly from one organism to another, but contained ammonium sulfate as the nitrogen source and glucose as the energy source in all cases. Appropriate inorganic salts and vitamins (where required) completed the medium, which was free of LBF. All organisms were incubated 3-4 days, and the activity determinations carried out on the culture filtrates.

† These crude media contained only small amounts (4 to 9 units per ml) of LBF before fermentation. Stillage is an aqueous residue of alcoholic fermentation; animal stick liquor is an infusion of slaughter house wastes which was obtained from Armour and Co.

TABLE II.
Comparative Rf Values of *Lactobacillus bulgaricus* Growth Factors Found in Yeast Extract and *Ashbya gossypii* Fermentation Products.

	Rf values				
	0.04	0.2	0.38	0.55	0.9
Difco yeast extr.	+	+	+	+	+
Ashbya gossypii culture filtrate	+	+			+
LBF conc. from *A. gossypii* culture filtrate					+

butanol was the mobile phase. When the strips were developed on agar plates seeded with *L. bulgaricus,* at least 3 zones of growth were observed. Chromatographs of yeast extract revealed 5 zones of which 3 were identical in Rf values to those contained in *A. gossypii* liquor. With LBF concentrated from fermentation liquor by a modification of an earlier described procedure(1) which involved adsorption on charcoal and elution with hot butanol, the same chromatographic procedure gave only one significant zone of growth, this having an Rf value of approximately 0.9. Rf values for the active substances present in the 3 materials are shown in Table II. It thus becomes apparent that several forms of the growth factor occur naturally, and that only one of these is concentrated by the procedure utilized above.

To determine the possible role of LBF in chick nutrition a concentrate of the factor was prepared(1) containing 108 units of activity per mg. By *L. leichmannii* assay this preparation was free of vitamin B_{12}. The chicks used were from the Hiram Walker flock of Hampshire males mated to Barred Rock females. The maternal diet, which resulted in chicks of low animal protein factor(s) storage, was an all vegetable protein base. In this experiment, the hatchability of fertile eggs was 46%.

Day-old chicks from the above hens were placed on the following basal ration: ground yellow corn 43.2, pulverized oats 10.0, wheat bran 3.0, wheat standard middlings 5.0, mineral mixture [steamed bonemeal 100, ground limestone 50, manganese sulfate 1.5] 3.0, iodised salt 0.5, soybean meal 35.0 and vitamin supplement [Viadex (5000A-1000D) 2,000, riboflavin 4, choline chloride 250, wheat standard middlings 1746] 0.3. To this ration the concentrate of LBF was added singly and in combination with fish solubles and crystalline vitamin B_{12} as shown in Table III. All chicks were reared in batteries with raised wire floors and with free access to water. The average weight gains after a 6-week period (Table III) show that the growth

TABLE III.
Six Weeks Gains of Chicks Fed Crystalline Vitamin B_{12}, Sardine Fish Solubles and LBF Concentrate.

Supplement (per 100 g of feed)	No. chicks		Avg 6 week responses (males + females)		
	Started	Died	g gain in wt	Gain over controls g	Gain over controls %
None	34	6	217	—	—
LBF conc. (.06 g)	34	3	242	25	11.5
Fish solubles (1 g)	34	4	440	223	103
LBF conc. (.06 g) and fish solubles (1 g)	34	1	483	266	123
Fish solubles (3 g)	34	6	519	302	139
LBF conc. (.06 g) and fish solubles (3 g)	34	2	546	329	152
Crystalline vit. B_{12}* (3 μg)	34	3	504	287	132
LBF conc. (.06 g) and crystalline vit. B_{12} (3 μg)	34	1	515	298	137

* Kindly furnished by E. R. Squibb and Sons.

rate of all groups receiving the LBF concentrate was increased. It is also of interest but of uncertain significance that death losses were lower where the LBF concentrate was included in the diet. Groups receiving the concentrate appeared better groomed than those that did not receive it, a factor which is not reflected in the more objective data recorded in Table III. Two other trials in which a concentrate of LBF was added to the above basal ration gave substantially the same growth response and general improvement in the appearance of the chicks.

The weight of recent evidence indicates that crystalline vitamin B_{12} does not fully duplicate the growth effects of crude sources of the "animal protein factor" when added to rations based upon vegetable proteins(3). The results of this and similar experiments are in accord with this view. Since 3% of sardine fish solubles consistently produces the maximum growth obtainable with this supplement in the basal ration used the further increase resulting from the addition of the concentrate of LBF points to the existence of a factor in this concentrate which promotes growth and seemingly is inadequate in fish products. Likewise, the somewhat lower response to vitamin B_{12} and to vitamin B_{12} combined with LBF concentrate in comparison to fish solubles indicates that the latter carry at least one growth-promoting substance other than vitamin B_{12}.

Conclusions. These studies substantiate and extend an earlier report regarding the wide distribution of the *Lactobacillus bulgaricus* factor in nature. In addition they reveal the possibility that this factor may be required for animal growth. Investigations on the biosynthesis, purification, and nutritional significance of this factor are being continued.

3. Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C. Jr., and Franklin, A. L., *J. Biol. Chem.*, 1949, v180, 647.

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