greater quantities than triiodothyronine(11-13). These observations have led to the suggestion that thyroxin is converted peripherally to triiodothyronine by a process of deiodination. In support of this possibility, Gross and Leblond found a compound, later identified as triiodothyronine, in the organs of thyroidectomized mice following the administration of I^{131} labelled thyroxin(1). These observations have been confirmed recently by Kalant *et al.* in propyl thiouracil-treated animals(3).

The data reported in this paper demonstrate that extrathyroidal conversion of thyroxin to triiodothyronine can occur, at least in the kidney.

Summary and conclusions. 1. Chromatographically pure I^{131} labelled l-thyroxin was added to surviving kidney slices which were incubated in a modified Krebs-Ringer phosphate solution. 2. Following incubation, the slices were homogenized, extracted with butanol and the thyroxin and triiodothyronine separated and identified by paper chromatography. 3. The triiodothyronine was found to be radioactive. This was taken as evidence of deiodination of thyroxin to triiodothyronine. 4. Conversion of thyroxin to triiodothyronine was reduced by excess iodide and abolished by boiling the tissue or by the addition of potassium cyanide to the media.

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Distribution and Properties of an Unidentified Growth Factor for Avian *Lactobacillus bifidus.** (21032)

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Veltre, Shorb, and Pelczar(1) reported that *Lactobacillus bifidus* of avian origin failed to grow in a defined medium unless supplied with a substance(s) found in Phytone, yeast extract, beef extract, or fish solubles. A disaccharide from mucin, galactose acetylglucosamine(2,3), and blood group substances A and B(4) failed to replace the requirement of the avian bifids as these compounds did for the variants of *L. bifidus* from humans. Because large numbers of the *L. bifidus* organisms were associated with a depression of chick growth(5) perhaps by robbing the avian host of some required nutrient, further studies on the distribution and properties of the substance required by these avian bifid organisms have been carried out.

Experimental. An assay procedure has

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TABLE I. Composition of Basal Medium.

	Amt/500 ml double strength medium
Cas amino acids	5 g
KH_2PO_4	2.5
Lactose	7
Sodium acetate (anhydrous)	5
Adenine, guanine, uracil, xanthine	10 mg each
$MgSO_4 \cdot xH_2O$	200 mg
NaCl, $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$	10 mg each
Ascorbic acid	2 g
Cysteine HCl	200 mg
Asparagine	100
p-Aminobenzoic acid	10 μg
Pteroylglutamic acid	10
Thiamine · HCl	200
Ca pantothenate	400
Nicotinic acid	600
Riboflavin	200
Pyridoxine HCl	1200
Biotin	4
Distilled water	500 ml
pH to 6.8	

been developed for the avian *L. bifidus* factor (ALbf), based on the observations of Veltre *et al.*(1) using poult strain T3 (ATCC 11,617). The composition of the basal medium is shown in Table I. All of the ingredients except the B vitamins and ascorbic acid were mixed and frozen. Just before use, the B vitamins, maintained as 2 stock mixtures(6) and ascorbic acid were added. The medium was adjusted to pH 6.8. The total volume in the 22 x 108 mm tubes was 6 ml, 3 ml being double strength medium and the remaining 3 ml consisting of sample and water. The assay tubes were autoclaved for 5 minutes at 120°C. The stock culture was transferred weekly on basal medium stabs containing supplements of 2% agar and approximately 50 mg of water soluble solids from fish solubles per 6 ml of single strength medium. The inoculum medium was of the same composition, with agar omitted. Because niacin was gradually destroyed by storage of the complete medium, the inoculum medium was made bi-weekly, autoclaved and frozen. It was thawed and warmed just before inoculation. The 24-hour inoculum culture was centrifuged once, and resuspended in physiological saline to give a galvanometer reading of about 85 on the Evelyn colorimeter, using the 620 mμ filter. One drop of inoculum was used per tube. The assay tubes, as well as the stock cultures and inoculum cultures were incubated aerobically. After 66 hours incubation, the assays were read by titrating to pH 7, using 0.05 N NaOH. A hot water extract of fish solubles (Gorton-Pew) was chosen as the standard. All samples were prepared as hot water extracts unless otherwise stated and all unit values were calculated on the basis of water soluble solids. The pH of the samples and standard was adjusted to 6.8 because *L. bifidus* has a very limited pH growth range. An excess of acid or alkali will completely prevent initiation of growth.

Results. A typical standard curve is shown in Fig. 1. The amount of fish solubles solids required for one-half maximum growth, when growth was measured by acid formation, was about 12.5 mg after 60 hours incubation. The assay organism produced rather limited amounts of acid, the highest titration values with optimum amounts of fish solubles being between 7 and 10 ml of 0.05 N acid. Doubling the amount of lactose in the medium did not improve the amount of acid formed. A steeper pitch in the slope of the standard curve was observed with the larger dosages of fish solubles or with some other samples, suggesting that the samples were carrying materials which were fermentable, giving rise to more acid. For calculating potencies of samples, only that part of the curve lying between

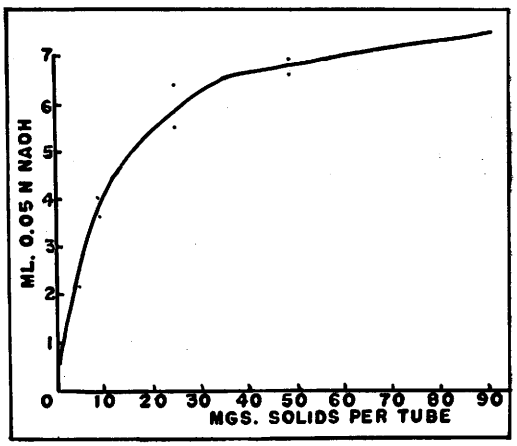

FIG. 1. Dosage response curve of *Lactobacillus bifidus* T3 (ATCC 11,617) with fish solubles standard, after 60 hr incubation.

TABLE II. Activity of Source Materials for Avian *Lactobacillus bifidus* Factor.

Samples (as hot water extracts)	Units*/mg of water soluble solids
Animal products and isolated proteins†	
Fish solubles	1.0
Crabmeal	3.1
Beef extract	1.7
Liver NF2 (Armour)	2.2
Chick heart	2.2
" livers	.3–1.6
" fecal contents (from 7 groups of chicks on casein-gelatin-cerelose diet, various supplements)	3.4–5.5
Plant products and isolated proteins†	
Phytone (Baltimore Biol. Lab.)	1.4
Alfalfa meal	1.6
Tomato pulp	2.5
Grass juice	3.0
Asparagus juice concentrate	3.6
Corn steep liquor	4.2
Fermentation products, bacterial cultures and yeasts†	
Penicillin mycelium residue	2.8
Soludri (Schenley)	2.9
Ethyl molasses solubles	2.8
Escherichia coli, parent strain,‡ 1-day incubation	4.8
Idem, 5-day incubation	1.4
E. coli, methionine-requiring strain,§ 1-day incubation	2.9
Aerobacter aerogenes, chick strain, Koser's citrate culture	2.2
A. aerogenes, poult strain, Koser's citrate culture	4.9
Bacillus subtilis preparations†	.6–20.0
" *megaterium* PYP 25	2.7
Yeasts and yeast products†	1.5–16.0

* 1 unit equivalent to 1 mg of water soluble solids from fish solubles.

† Egg albumin, egg yolk, Hi Pro (hydrolyzed chicken feathers), gelatin, whey, casein, trypsinized casein, trypsin, pepsin, pancreatin, Mylase P, fishmeal, whale solubles, molasses, soybean meal, alpha protein and Dracket protein, contained less than 1 unit/mg water soluble solids. Of the 16 *B. subtilis* cultures and preparations tested, 1466A and 1466B had activities of 20 and 14 units/mg respectively. Of the 14 yeast preparations tested, one preparation of *Candica flarari,* Pabst 186-819D, had 16 units/mg.

‡ Parent strain(7) of *E. coli,* kindly supplied by Dr. Esther Stubblefield, The Upjohn Company. It was grown on Koser's citrate broth, with 0.5% glucose.

§ Grown on methionine assay medium, with methionine added.

2 and 7 ml of acid was used. One mg of water soluble solids from fish solubles was adopted as one unit of ALbf. Day to day reproducibility was rather good with the fish solubles standard but a trial standard prepared from Pabst yeast R_1 was found to lose about half its activity over a 6-week period, during which time the preparation was kept frozen. Fish solubles from different sources and batches varied in ALbf content. Some samples, especially those with rancid odor contained inhibitory substances.

Table II shows the distribution of ALbf in various source materials of animal and plant origin, in fermentation products, yeasts, and bacterial cultures. Isolated proteins, milk products and some animal products have a rather low content of ALbf, less than one unit per mg of solids. Fish products, chick organs, liver NF2, and some plant materials, have slightly more activity, between one and 3 units per mg. The cecal content of chicks, however, had a higher value, between 3.4 and 5.5 units per mg, in the same range of activity as extracts of vegetables and fermentation products, such as corn steep liquor, asparagus juice concentrate, grass juice extract, penicillin mycelium residue, and distillers solubles. The high content of ALbf in products produced by microbial activity suggested that pure cultures of microorganisms might contain the factor. Whole cultures of *Escherichia coli*(7) and *Aerobacter aerogenes,* grown on Koser's citrate-glucose broth, itself inactive, produced from 1.4 to 4.9 units of ALbf per mg. One culture of *Bacillus megaterium* and a series of *Bacillus subtilis* culture preparations contained ALbf in amounts ranging from 0.6 to 20 units per mg. A series of yeasts and yeast products showed activity from 1.5 to 16 units per mg. Materials found to be inactive, in addition to those previously reported(1) were yeast nucleic acid and strepogenin(8), using fraction A(9) which is active for *Lactobacillus bulgaricus* 09. With the exception of whey, the distribution of ALbf showed a similarity of distribution with those materials containing unidentified chick growth factors. The chick growth factors have been reported in whey, fish meal, fish solubles, liver, grass juice, alfalfa, distillers solubles, various yeasts, *A. aerogenes, E. coli,* and *B. subtilis.* The work on the distribution and fractionation of these chick factors has recently been reviewed(10-19). It was therefore of interest to determine whether the factor required by

TABLE III. Activity of Fish Solubles,* Liver† and *Escherichia coli* Fractions for *Lactobacillus bifidus* and Chicks.

Sample	Conc. of *L. bifidus* activity	Conc. of chick activity
Fish solubles	1.0	1‡
Idem, hot water extr.	1.3	
" , fraction M12	1.0	38‡
" , " M26	1.3	190‡
Liver NF2	2.2	1§
Idem, phenol-butanol solvent fraction	6.6	58§
Liver Biopar C, counter current tube 4	.0	340§
Idem, tube 8	1.3	0§
E. coli, whole culture	2.9	Active§
Idem, ethanol eluate from Norit A	.4	" §

* Fractions M12 and M26 kindly supplied by Dr. Henry Menge, Poultry Husbandry Division, Agricultural Research Center, Beltsville, Md.

† Fractions from liver NF2 and Biopar C prepared by Dr. Henry Menge, Dr. G. H. Arscott, and Dr. G. F. Combs, Poultry Husbandry Department, Univ. of Md.

‡ Activity based on organic solids, data supplied by Dr. H. Menge.

§ Data supplied by Dr. H. Menge, Dr. G. F. Combs, Dr. G. H. Arscott and Dr. G. L. Romoser, Poultry Husbandry Department, Univ. of Md.

L. bifidus was identical with any of the chick growth factors. Potent chick fractions were obtained and tested for *L. bifidus* activity.

Table III shows the activity of the chick fractions for *L. bifidus* and for the chick. The chick activity has been expressed in terms of concentration during fractionation. Fraction M12 from fish solubles showed increased activity for the chick but not for *L. bifidus*. Fraction M26, very active for the chick, had low activity for *L. bifidus*. A series of fractions from liver NF2 and liver Biopar C were also tested. Liver NF2 was somewhat more active than fish solubles for *L. bifidus*. *L. bifidus* activity followed, to some extent, the chick activity through the phenol-butanol solvent procedure, but it did not follow the chick activity after counter current distribution. The chick activity was concentrated in tube 4, which was inactive for *L. bifidus*, while tube 8 contained some *L. bifidus* activity but was inactive for the chick. An *E. coli* culture and the alcoholic eluate from a Norit treated culture of *E. coli*, both having activity for the chick(20), had some *L. bifidus* activity, although the eluate had lower ALbf content than the original culture. Whey had low ALbf content and no fractions prepared from whey increased *L. bifidus* growth. No concentrates of the grass juice or alfalfa chick factors have been available for testing. The distribution of ALbf coincides with the distribution of these factors in some products.

The possibility existed that fractionation of the chick growth factors might destroy a part of ALbf, if ALbf were multiple in nature. Therefore various inactive substances such as extra casein, egg yolk, egg albumin, vitamins, and growth factor supplements have been tested with M12 or similar ALbf-inactive chick-active fractions, without producing growth of *L. bifidus*. Galactose-acetylglucosamine added to various ALbf-inactive chick-active fractions did not result in growth of *L. bifidus*. Growth was depressed slightly when the disaccharide was added to active ALbf fractions. Before embarking on a fractionation program it was of interest to determine some of the chemical and physical properties of the factor using fish solubles as the source material. The activity was soluble in phenol or water and in laboratory grade methanol, tertiary butanol, and ethylene glycol monomethyl ether. It was soluble in ethanol-water and ethylene glycol monobutyl-water solvent systems, but not soluble in petroleum ether, ethyl ether, absolute ethanol, acetone, chloroform, carbon tetrachloride, toluene, n-butanol, benzene, pyridine, ethylene glycol monobutyl ether, dioxane, caprylic alcohol or benzyl alcohol. It was absorbed on supercel, Norit A and alumina. It was eluted from Norit A by phenol, methanol-water, ethanol-water, and the cellosolve water systems. The activity was not eluted from alumina by any of the solvents tested. Combinations of alumina eluates were not active. The factor acted as a cation on paper electrophoresis and it was dialyzable. The growth factor, contained in a phenolic eluate from Norit A, moved as one elongated unit on paper chromatograms in a 60% ethylene glycol monobutyl ether-water solvent system, the Rf value being between 0.4 and 0.7. A rechromatographed sample lost activity completely, suggesting separation of factors or destruction of activity. The activity disappeared on treatment with potassium perman-

ganate, lead acetate and silver nitrate. All the fractions from these last 3 treatments contained inhibitors, in a plate assay, so that activity could be masked by toxic substances. In this respect, phenol fractions also contained inhibitors. ALbf activity was not destroyed by autoclaving at either pH 0.9 or pH 10 for 30 minutes at 15 lb pressure, by standing from 30 minutes to 3 hours at room temperature with 6 N HCl, nor by exposure to ultraviolet light for one hour. It was partially destroyed by 15 minutes exposure to concentrated nitric acid at room temperature, by autoclaving at 15 lb pressure for 30 minutes at pH 12 and by exposure to sunlight for one week. The activity was completely destroyed by refluxing for one hour with 6 N HCl. Ash from fish solubles was inactive.

Various procedures have been tried in concentrating the growth factor. Approximately 6- to 10-fold increases in activity have been obtained in 70% ethanol or 60% ethylene glycol monobutyl ether eluates from Norited water extracts of yeast R_1 and fish solubles. No concentration was obtained in the isobutyl alcohol precipitates of fish solubles by the method described by Weise *et al.*(21) for the chick growth factor in fish solubles. The precipitate was inactive for *L. bifidus* and the activity remained in the water layer of the 80% isobutyl alcohol-water mixture. Further work is in progress on fractionation of the *L. bifidus* factor.

Two branched bifids of human origin (ATCC 11,146 and 11,147) and 2 unbranched strains of human origin (ATCC 4962 and 4963) originally classified as *L. bifidus* but now classified as *L. acidophilus,* have been used as assay organisms in the ALbf assay. None of these cultures would grow with yeast R_1 at 2.85 mg per 6 ml, the amount causing optimum growth of the avian bifids. Perhaps these cultures would grow in the presence of galactose acetylglucosamine, the disaccharide from mucin, but this was not tested. The mutants of human *L. bifidus* requiring this disaccharide(2,3) were not available for use in our assay.

Discussion. The avian *L. bifidus* factor is not identical with that factor reported by György and associates(22-25) in human milk, which promotes the growth of human *L. bifidus* variants. The substances which influence the growth of the human variants, galactose acetylglucosamine(2,3) blood groups substances A and B(4) as well as pantethine(3) and ammonic compounds(4) were inactive as ALbf. The distribution of the 2 factors is entirely different, yeast extract and vegetable extracts being inactive for the human bifids(3,22) while very active as ALbf.

Although no positive identity has been established between ALbf and the liver, whey, *E. coli, B. subtilis* or fish solubles chick growth concentrates, the common distribution of factors suggests that further work may establish some relationship. Lack of correlation is not surprising in view of the recent unpublished work in this department(26) and elsewhere (27) indicating that 2 or more factors are acting together to influence chick growth. The low content of ALbf in casein, gelatin and Dracket protein would suggest that these ingredients might be used in a basal diet in developing a chick assay for *L. bifidus* factor. Such tests are now being tried in this department. Success in such tests may depend on the amount of intestinal synthesis of ALbf, because cecal contents have been demonstrated to be potent sources of the ALbf activity.

Summary. A method of assay for a growth factor required by *L. bifidus* of avian origin has been developed. The factor is found in low amounts in isolated proteins and animal products, in higher amounts in plant materials, and in highest amounts in fermentation products, yeasts and bacterial cultures. The growth factor was not found in concentrates of liver, whey and fish solubles which were potent sources of unidentified chick growth factors. Chemical and physical properties of the *L. bifidus* factor are described.

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Effect of β,β-Diethylalanine on Composition of Rat Livers. (21033)

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The effects of oral and intraperitoneal administration of β,β-diethylalanine (DEA) on growth and liver composition in rats has been studied with the view that it might be an antagonist of valine, isoleucine, or leucine. An antagonism involving one of these amino acids would be particularly advantageous for studying protein synthesis because, unlike methionine and tryosine, these amino acids are not known to be concerned with anabolic reactions aside from those occurring in the synthesis of protein molecules.

Materials and methods. Synthesis of DL-DEA. A modified malonic ester synthesis has previously been employed for the preparation of DL-DEA(1). The material for these experiments, however, was synthesized by HCl hydrolysis of the corresponding hydantoin (m.p. 182°) prepared by the general hydantoin synthesis of Bucherer *et al.*(2,3). The HCl was removed on an amberlite IR4-B ion exchange column. An overall yield of 41% was obtained. The product was identified by synthesis of the chloracetyl derivative(1) which gave the theoretical neutralization equivalent [m.p. 130°; DuVigneaud *et al.*(1) 127-128°]. On paper chromatograms the free amino acid gave a single ninhydrin positive spot. *Feeding of DL-DEA.* The control diet A contained vitamin-free casein (Labco), 10;