TABLE II. Recovery of I^{131} in the Presence of Tissue.

Tissue	Wt of sample, g	Recovery of I^{131}, %
Skin	1	101.7
Adipose tissue	1	98.1
Liver	1	99.3
Spleen	1	90.6
Kidney	1	96.5
Adrenal	.073	96.5
Ovary	.053	116.2
Pituitary	.014	99.7
Brain	1	100.6
Smooth muscle (ileum)	1	96.4
Striated muscle	1	91.5
Blood	1	92.3
Thymus	.55	98.5
Thyroid	.017	93.7
Feces	1	93.1

various tissues was determined by the addition of 1 cc of a solution containing .008 microcurie of I^{131} and 0.1 μg of I^{127} per cubic centimeter to 15 tissues of the rat. Various tissues were chosen because the presence of volatile substances in certain tissues might easily affect the recovery of I^{131}. The results of this experiment are shown in Table II, which indicates that satisfactory recovery was achieved in each instance in spite of the fact that only one sample was determined.

Comment. The procedure just described is an adaptation and a modification of the Chaney method for the chemical determination of iodine in biologic material with a few changes necessary for isotope measurement. The method has proved to be useful because of its convenience and sensitivity. Twenty samples can be digested and distilled conveniently and the radioactivity determined the following day. Because of the small amount of solids in the distillate, very satisfactory planchettes† always result, and corrections for self-absorption are unnecessary. The over-all sensitivity of this method was computed to be 30% by comparison with a uranium beta ray standard, that is, 30% of the beta radiation of the sample is recorded by the apparatus. With this sensitivity, as little as 3 x 10^{-5} microcurie of radioiodine per cubic centimeter of serum may be determined accurately at a counting rate 10 times the background. It is therefore possible to determine the concentration of I^{131} in serum of patients during a half-life of I^{131} following administration of doses of about 20 microcuries of radioiodine.

Summary. A convenient and accurate method for the measurement of small amounts of I^{131} in biologic material is described.

† For planchettes nickle-plated cups 2.5 cm in diameter and 0.25 cm deep are used. These are placed in a brass holder at a distance of 8 mm from the Geiger-Müller tube. The Geiger-Müller tube is shielded throughout by an inch of lead. With this shielding the cosmic and incidental radiation background varies from 0.2 to 0.3 count per second. The shielding for the Victoreen tube was designed by Dr. M. M. D. Williams, Division of Biophysics and Biophysical Research, and Mr. Dana Rogers, Division of Engineering, Mayo Clinic, to both of whom the authors are greatly indebted.

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Replacement of Pantothenic Acid by the *Lactobacillus bulgaricus* Factor in Rat Nutrition.* (18209)

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(Introduced by G. Howard Satterfield)

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The existence of a factor essential for the growth of *Lactobacillus bulgaricus* (Sarles) and *Lactobacillus helveticus* 80 has been re-

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TABLE I. Mean Weight Gains in Rats on Diets Supplemented with Calcium Pantothenate and LBF Concentrate.

Diet No.	Daily supplement	No. of survivors	Gain in wt, g			
			5 days	7 days	10 days	12 days
1	None	0	—	—	—	—
2	Ca pantothenate 12.5 μg	4	8 ± .9*	10.5 ± .7	16 ± 1.2	21.3 ± 3.3
3	" " 25 "	6	10 ± .5	12.3 ± .6	20.7 ± 1.4	27 ± .9
4	" " 50 "	8	10.3 ± 1.0	13.9 ± .6	19.4 ± .8	29.5 ± .8
5	" " 100 "	8	11 ± .3	15.4 ± 1.0	18.5 ± 1.4	30.8 ± 1.3
6	" " 200 "	8	11.5 ± .7	16.2 ± 1.0	20.5 ± .9	30.4 ± .9
7	LBF concentrate 3200 units† (equivalent to 96 μg pantothenate)	8	10.2 ± .8	17.1 ± 1.3	18 ± 1.3	29.3 ± .8
8	LBF conc. 6400 units (equivalent to 192 μg pantothenate)	8	9.5 ± .6	16.1 ± .4	16.8 ± 1.2	29.1 ± 1.0

* Mean ± stand. dev. of the mean.

† The unit of LBF is defined as that amount contained in 1 mg of standard Basamin-Busch yeast extract(1).

ported recently(1). This factor (LBF) has subsequently been shown to be essential for several other strains of the more fastidious lactic acid bacteria(2,3) and to be interchangeable with pantothenate for *Lactobacillus casei* and *Lactobacillus arabinosus*(4).

Partial identification indicates that LBF contains pantothenate in a bound form that is 50 to 100 times more active than free pantothenate for organisms which require LBF (4). Rasmussen *et al.*(5) have suggested that LBF may be required for the maximum growth of chicks on a vegetable-protein basal diet supplemented with fish solubles and vitamin B_{12}. This study was undertaken to determine the ability of the rat to utilize LBF on pantothenate-deficient diets.

Experimental. Rats of the Wistar strain, 22 to 24 days old, were placed on a basal diet consisting of 65% glucose, 18% casein, 10% fat, 4% minerals (USP No. 2), 2% cellulose, and 1% cod-liver oil. This was supplemented with 20 μg each of thiamine, riboflavin, pyridoxine, and 5 mg of choline chloride per rat per day. This is essentially the diet of Unna(6) and has been found adequate in our laboratory when supplemented with pantothenic acid. Rats on this regimen lost weight at an average of 0.5 g per day during the depletion period of 21 to 23 days. Prior to feeding the test diets, rats were selected as to weight and sex and placed in individual cages on wire floors. Graded doses of calcium pantothenate and two levels of LBF concentrate were fed in addition to the basal diet and vitamin supplement. The vitamin supplements and the test levels of calcium pantothenate or LBF concentrate were mixed with the basal diet in such proportion that 2 g contained the daily dosage. The supplemented test diets and unsupplemented basal diet were weighed out to the rats daily in flanged cups to prevent spillage. Uneaten food was weighed and the amount of unsupplemented basal diet fed was adjusted to insure daily ingestion of the supplemented test diet. Eight rats, 4 males and 4 females, were fed each test diet. Four rats, 2 males and 2 females, served as controls. The rats were weighed at intervals of 2 to 3 days and the gains are recorded in Table I. The LBF concentrate was prepared from *Bacillus megatherium* culture filtrate by adsorption on charcoal and elution with *n*-butanol.

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

2. Kitay, E. and Snell, E. E., *J. Bact.*, 1950, v60, 49.

3. Wood, H. N. and Williams, W. L., unpublished data.

4. McRorie, R. A., Masley, P. M., and Williams, W. L., *Arch. Biochem.*, 1950, v27, 471.

5. Rasmussen, R. A., Smiley, K. L., Anderson, J. G., Van Lanen, J. M., Williams, W. L., and Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 658.

6. Unna, K., *J. Nutrition*, 1940, v20, 565.

It contained 38,500 LBF units per ml and had a potency of 840 units per mg of dry solids. Diet 7 contained 3,200 LBF units in a daily supplement and was equivalent to 96 μg of bound calcium pantothenate as determined by alkaline hydrolysis and microbiological assay(7) of the β-alanine liberated. Paper chromatography of materials containing LBF has shown that 5 or more substances possess LBF activity(5,8). The concentrate used in this study consists mainly of a mixture of the 2 LBF's (Rf 0.83 and 0.74) obtained in concentrates from *B. megatherium* culture filtrates(8). Less than 2 μg of free pantothenate were contained in a daily supplement. Diet 8 contained twice these amounts of free pantothenate and pantothenate bound in the LBF molecule.

Results and discussion. There was no significant difference between the growth responses produced by the 4 highest levels of calcium pantothenate and either level of LBF concentrate at the end of 12 days. This merging of response is to be expected since a daily dose of 25 to 50 μg of Ca pantothenate appears to be adequate for optimum growth after the rat has recovered from severe depletion(6).

Diet 8 had a pronounced odor and was not readily consumed by the rats. Subnormal gains on this diet were probably the result of inadequate food intake due to unpalatability, although the possibility of toxicity due to impurities in the concentrates must also be considered.

Controls and some of the rats on the low pantothenate diets died in 3 to 4 days after the start of the experiment, as shown in Table I. All rats receiving over 50 μg of calcium pantothenate or the LBF supplements survived. This leaves little doubt as to the replacement of pantothenate by LBF for the rat. Further proof of this interrelationship is evidenced by the fact that rats which were prostrate after a depletion of 24 to 25 days were on their feet and had apparently recovered in a few hours after intraperitoneal injection with LBF concentrates or calcium pantothenate. Death occurred in 1 to 2 hours after prostration in uninjected rats.

Summary. Evidence is presented to show that concentrates of the *Lactobacillus bulgaricus* factor will replace pantothenic acid in the nutrition of the rat. The quantitative effect of LBF in promoting growth may be in excess of that expected from the pantothenate contained in the LBF molecule.

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7. Atkin, L., Williams, W. L., Schultz, A. S., Frey, C. N., *Ind. Eng. Chem. Anal. Ed.*, 1944, v16, 67.

8. Long, C. L., and Williams, W. L., *J. Bact.*, in press.

Occurrence of Tyrosinase in Horse and Fish Melanomas. (18210)

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In addition to the presence of melanomas in man, these tumors have been reported in the horse, mule, cow, swine, goat, dog, cat, chicken, rabbit, mouse, snake, and fish(1-6). De-

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1. Feldman, W. H., Melanoblastoma. *Neoplasms of Domesticated Animals*, Philadelphia, W. B. Saunders Company, 1932, chap. 16, pp 247-268.

2. Hadwen, S., *Canad. M. A. J.*, 1931, v25, 519.

3. Mathews, F. P., *J. Am. Vet. M. A.*, 1929, v74, 1071.