animals had myocardial or renal pathology that could be considered to be more than slight.

The reason for these results is not known. Rix and Ebrhardt(4) observed fluctuations in the potassium and calcium concentrations in the blood sera of rabbits when linseed oil was added to the rabbits' hay and turnip diet as daily 5 cc doses. They believed that the increase in serum potassium which they observed during the first 6 weeks of linseed oil administration was due to increased absorption of potassium from the intestine. They, however, were unable to explain the rapid fall in serum potassium concentration which occurred in their rabbits after the first 6 weeks on this regimen. The potassium content of the diet which they employed was not stated.

Summary. A diet containing 0.03% potassium and 20% corn oil produced a greater incidence and extent of the myocardial necrosis and fibrosis and dilatation of renal tubules typical of potassium deficiency than did an isocaloric, 5% corn oil diet containing the same amount of potassium when the 2 diets were pair fed. No statistically significant differences were observed in other criteria of potassium deficiency.

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Received October 7, 1952. P.S.E.B.M., 1952, v81.

Evidence for an Unidentified Growth Factor Required by *Microbacterium flavum.** (19892)

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(Introduced by George M. Briggs.)

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Doetsch and Pelczar(1) reported that several strains of *Microbacterium lacticum* would grow in a medium consisting of vitamin-free acid-hydrolyzed casein, inorganic phosphates, calcium D-pantothenate and thiamin hydrochloride. Calcium D-pantothenate was shown to be essential for growth and thiamin to be stimulatory. The same workers(2) reported similar results when 18 amino acids were substituted for the casein hydrolysate. Supplementation of this latter medium with riboflavin, niacin, pyridoxine, biotin, folic acid, and glutamine produced growth comparable to that obtained in trypticase soy broth. *Microbacterium flavum,* however, was unable to grow in any of the synthetic media prepared. Bishop *et al.*(3) substantiated these results with *M. flavum* and found that addition of crude materials such as phytone, yeast extract, proteose-peptone No. 3, Wilson's Liver L, and asparagus juice resulted in media which supported good growth of *M. flavum.* The data indicated that a combination of substances was active in stimulating optimum growth. A further study of the nutritional requirements of this organism is the subject of this report.

Methods. Microbacterium flavum, OJ8, (ATCC 10340), was maintained on slants of Difco Micro Culture Assay Agar. Subcultures were made weekly, incubated for 24 hours at 30°C and then stored in the refrigerator. Inocula were prepared by transferring the stock cultures to 10 ml of Difco Micro Inoculum Broth and incubating at 30°C for 24 hours. The culture was centrifuged, washed

* Scientific paper No. A378. Contribution No. 2389 of the Maryland Agriculture Experiment Station, Department of Poultry Husbandry, College Park, Md. This study was supported in part by a grant from Merck & Co., Rahway, N. J.

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TABLE I. Effect of EDTA and $MgSO_4$ on Growth of *M. flavum*.

$MgSO_4$, mg./tube	Galvanometer readings: No EDTA	Galvanometer readings: 300 μg EDTA/tube
0	94	100
1	81	100
5	67	85
10	62	76.5
50	56.5	68
100	56	63.5
200	56	
500	80	100

twice with physiological saline, resuspended in 10 ml of saline and adjusted by dilution to a reading of 65 on the Evelyn Photoelectric Colorimeter, using the 515 mμ filter. One drop of a 1:100 dilution of this suspension was used to inoculate each tube of experimental media. The basal medium employed was that of Doetsch and Pelczar modified by omission of glutamine, hydroxy-L-proline and DL-threonine and addition of aspartic acid (0.05%) (3), and $MgSO_4 \cdot 7\ H_2O$ (0.0033%). Samples of materials tested for growth response were added to 20 × 150 mm tubes in the desired amounts in duplicate, the volume made to 3 ml with distilled water and 3 ml of the medium (double strength) added, giving a total volume of 6 ml per tube. These tubes were then capped with glass hats, autoclaved for 12 minutes at 120°C, cooled and inoculated. They were incubated at 30°C for 36-38 hours and were agitated daily to facilitate reading by breaking up the clumps of bacteria. The amount of growth was determined by means of the Evelyn Photoelectric Colorimeter using the 620 mμ filter. Final pH and acid titration of individual tubes did not follow dosage response patterns. A standard reference material was prepared by hydrolyzing soy bean oil meal (Archer-Daniels-Midland Co.) with 12 N HCl, adsorbing with Norit at pH 3.5 for 6 hours (on the basis of 1 g of Norit to 1 g of dry hydrolysate), and neutralizing. The filtrate standard was designated S3, and 1 mg was arbitrarily assigned one unit. A dosage response curve was obtained with 2 to 100 mg S3 per 6 ml of medium. A similar fraction, P3, was prepared from Alpha protein (The Glidden Co.), a com-

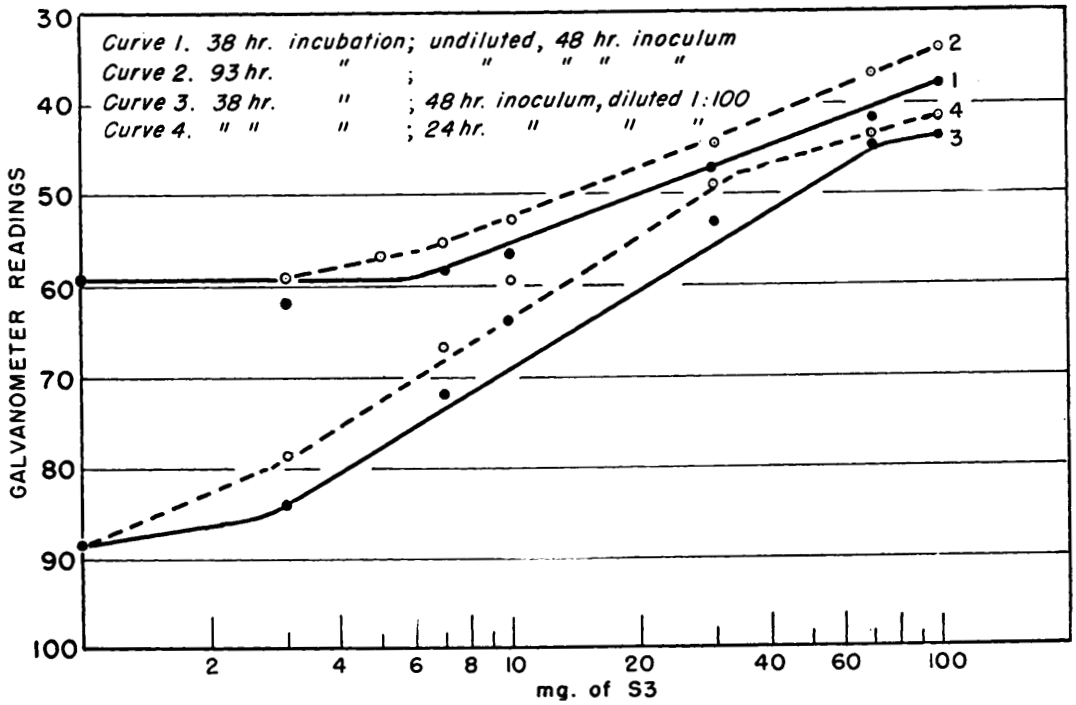

FIG. 1. Effect of varying inoculum and incubation time on growth response curves for S3 in the presence of $MgSO_4 \cdot 7H_2O$.

mercial preparation of the protein portion of soy bean meal.

Results and discussion. In preliminary work the testing of a variety of compounds indicated a requirement for minerals. Consequently $NaSiO_3$, $CaCl_2$, Na_2SO_4, NaCl, $MgSO_4$, $FeSO_4$, $SrCl_2$, $MnSO_4$, and Na_2SO_3 were tested for activity in the medium lacking both $MgSO_4$ and a source of S3 and with a 48-hour inoculum merely adjusted to 65 on the colorimeter without the 1:100 dilution. Only $MgSO_4$ stimulated growth and that only to the half-maximum point. Addition of EDTA (ethylene diamine tetraacetic acid, a solubilizing chelating agent) caused complete inhibition which was overcome by addition of $MgSO_4 \cdot 7\ H_2O$ (Table I). Thus $MgSO_4$ was included in the medium in the concentration indicated above. The assay procedure was further improved by reducing the inoculum growth period from 48 to 24 hours, reducing the assay incubation time from 96 to 48 hours and by diluting the inoculum 1:100. The effects of these are shown in Fig. 1, using S3 as the sample. It will be noted that little growth was obtained when no sample was added, steeper curves resulted on addition of S3 and straight line responses were easily obtained on semi-logarithmic graphs when the inoculum was diluted 1:100. The growth response obtained with the inclusion of optimal amounts of S3 in the medium was comparable to that obtained with trypticase soy broth or Difco Micro Inoculum Broth.

Using the improved medium and inoculum, a recheck of the source materials previously tested (*v. supra*) showed that Wilson's liver L, asparagus juice, P3 and yeast extract were still active while phytone, though stimulatory, was less active than before (Table II). This indicated that part of the original activity of phytone was probably due to the presence of magnesium. The lack of complete growth at high levels of phytone may be due to a high magnesium content and resultant toxicity (Table I), or the higher activity of the other materials may be due to a larger concentration of a secondary growth-stimulating factor.

Since good responses were obtained with the hydrolyzed protein, S3, and since S3 was Biuret negative, 20 amino acids individually and in several combinations and vitamin-free casein hydrolysate were tested for effect. None of these stimulated growth in the presence or absence of S3. This is not surprising since P3, the preparation from Alpha protein, had only 40% the activity of S3.

TABLE II. Potencies* of Source Materials for *M. flavum*.

Sample	Units/mg
Phytone	.3
P3†	.4
S3†	1
Yeast extr.	1
Liver L	2.8
Asparagus juice	3.8

* 1 unit = 1 mg of the Norit filtrate of the HCl hydrolysate of soy bean meal.
† See text for description.

The active factor(s) could not be replaced by the *Leuconostoc citrovorum* factor, a *Lactobacillus bulgaricus* factor concentrate, vit. B_T concentrate, fraction A (a peptide fraction from hemoglobin isolated by Kao, unpublished), orotic acid, vit. B_{12}, biocytin, cytidylic acid, xanthine, adenine, guanine, uracil or a combination of the last three. The factor(s) is not strepogenin since it is not a peptide, it withstands HCl hydrolysis(4) and activity remains even after 4 Norit adsorptions, which procedure removes strepogenin(5).

Other characteristics of the factor(s) found in S3 are stability to treatment with HNO_2 and H_2O_2, appearance on both sides of a dialyzing membrane with slightly more activity residing in the residue and separation from aqueous solution by acetone. After addition of only a small amount of acetone an immiscible brown liquid which contained most of the activity appeared at the bottom of the container. Even though there was a decrease in total weight there was not a proportionate increase in activity, possibly indicating the presence of an inhibitor.

Studies with paper partition chromatography indicated that further purification and concentration were necessary since the large sample required to obtain a growth response caused streaking of the chromatograms.

Summary. *Microbacterium flavum* has been shown to require an unidentified factor(s) and

magnesium for optimal growth in a synthetic medium. The addition of this substance to an otherwise synthetic medium supports growth approximately equivalent to that obtained in Difco Micro Inoculum Broth or trypticase soy broth. The unidentified factor(s) can be found in Wilson's liver L, asparagus juice, yeast extract, soybean meal, and Alpha protein. It is not replaceable by any known growth factors tested. It is stable to HCl hydrolysis, treatment with HNO_2 and H_2O_2 and is separated from aqueous solution with acetone.

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Received October 7, 1952. P.S.E.B.M., 1952, v81.

Intestinal Absorption of Glucose in Hypothalamic Obesity. (19893)

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The demonstration that the obesity which often appears in rats as a result of damage to the hypothalamus is primarily due to hyperphagia(1) has not entirely dispelled the traditional concept that this disorder may be a manifestation of some disturbance of metabolism. Among the more commonly indicted metabolic processes are those involved in intestinal absorption. Soulairac(2) has reported that hypothalamic lesions in rats may elevate the rate of glucose absorption from the intestine. Unfortunately, his data are too few for statistical analysis, and although he claimed that changes in the rate of glucose absorption run parallel with changes in the appetite for food (and specifically for glucose) he failed to show that the animals in which he found an increased rate of glucose absorption (10 days after placing the lesions) were either hyperphagic or obese. The relationship of Soulairac's finding to hypothalamic obesity, therefore, appears doubtful. On the other hand, Brooks(3) concluded, on the basis of indirect evidence (food-feces ratios), that the rate of intestinal absorption of food in rats with hypothalamic obesity was not elevated above that in controls.

The present investigation was undertaken since direct observations of the rate of glucose absorption in rats with clearly established hypothalamic obesity have not heretofore been reported.

Experimental. Large bilateral electrolytic hypothalamic lesions were placed in a series of male rats (of both Sprague-Dawley and Long-Evans strains, weighing between 220 g and 300 g) using the Krieg-Johnson* stereotaxic apparatus. The rats were operated upon in several groups, a few non-operated rats from each group being reserved as controls. The animals were then kept in individual cages for periods ranging from 29 to 47 days and fed Rockland chow pellets *ad libitum*. Twenty-one rats were used for glucose absorption determinations: 10 unoperated controls, 9 with hypothalamic obesity (HO), and 2 HO thyroxinized.† In addition, 3 unlesioned rats were thyroidectomized (3-4 weeks prior to use) and five were rendered hyperthyroid† to serve as method controls. The determination of glucose absorption was carried out on anesthetized rats exactly as described by Bogdanove and Barker(4). Following a 48-hour fast the rats were given nembutal (45 mg/kg)

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* Obtained from the Johnson Scientific Co., Berwyn, Ill.

† Thyroxine was injected subcutaneously in 200 μg doses thrice daily for 7 to 11 days.