TABLE IV.
Effect of Carbohydrate in Producing a Growth Response to Vitamin B_{12}.

Ration	Avg 3 week growth, g
Sucrose-casein basal + 0.15% iodinated casein	60
" " " + " " " + 0.5 μg B_{12}/day	69
" " " + 0.25% " "	54
" " " + " " " + 0.5 μg B_{12}/day	65
Dextrin-casein basal + 0.15% " "	66
" " " + " " " + 0.5 μg B_{12}/day	93
" " " + 0.25% " "	76
" " " + " " " + 0.5 μg B_{12}/day	90
Corn starch-casein basal + 0.25% iodinated casein	77
" " " " + " " " + 0.5 μg B_{12}/day	87
Sucrose-casein basal + 45% defatted corn meal + 0.25% iodinated casein + 0.5 μg B_{12}/day	85
Sucrose-casein basal + 2% extracted corn fat + 0.25% iodinated casein + 0.5 μg B_{12}/day	73

Ershoff(2) has reported an antithyrotoxic factor present in extracted liver residue; however, the exact nature of this substance or its origin is not known. The observed phenomenon with liver powder might possibly be attributed in part to the liver glycogen since activity was reported only with the residue fed at a 10% level. Altering the carbohydrate of the ration may function in producing a more favorable synthesis within the intestinal tract of other required factors.

Summary. The failure of rats to respond completely to vit. B_{12} on a sucrose-casein diet containing a thyroid active material can be overcome by substituting defatted corn meal, corn starch or dextrin for the sucrose. The nature of this effect may possibly be attributed to the intestinal synthesis of other required factors. An explanation for the counteractive effect of corn oil on the growth depression in the hyperthyroid rat is offered.

We are indebted to Merck and Co., Inc., Rahway, N. J., for crystalline vitamins including B_{12}, and to Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic folic acid.

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Role of Protogen in the Nutrition of an Unidentified Corynebacterium (17975)

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The protozoon *Tetrahymena geleii* has been shown by Dewey(1) to require an unknown growth factor. Some of the properties of this factor were described by Stokstad *et al.*(2) and the name "protogen" was suggested. Kidder and Dewey(3) have shown that *T. geleii* will grow rapidly on a chemically defined medium with the addition of relatively small amounts of concentrates of protogen. We wish to report the findings of a bacterium which also requires protogen for growth.

Experimental and results. The organism* is a gram positive bacillus, 0.2 by 1.0μ, isolated from a sample of cow manure. It grows under aerobic conditions on a wide variety

1. Dewey, V., *Biol. Bull.*, 1944, v87, 107.
2. Stokstad, E. L. R., Hoffman, C. E., Regan, M., Fordham, D., and Jukes, T. H., *Arch. Biochem.*, 1949, v20, 75.
3. Kidder, G. W., and Dewey, V., *Arch. Biochem.*, 1949, v20, 433.

* We wish to thank Dr. M. A. Petty of Lederle Laboratories for the culture of this organism.

TABLE I.
Composition of Medium.

	Amt per liter of final medium
Acid hydrolyzed casein	5 g
Dextrose	5 "
Sodium acetate (anhydrous)	2.5 "
Speakman salt solution A	5 ml
" " " B	5 "
Asparagine	50 mg
DL-tryptophane	100 "
L-cystine	100 "
Adenine	10 "
Guanine	10 "
Uracil	10 "
i-inositol	5 "
Choline chloride	5 "
Calcium pantothenate	.5 "
Thiamine	.5 "
Nicotinamide	.5 "
Riboflavin	.5 "
Pyridoxine	.5 "
P-aminobenzoic acid	.5 "
Pteroylglutamic acid	.5 "

of carbohydrates. These carbohydrates include arabinose, galactose, glucose, mannose, rhamnose, xylose, lactose, maltose, sucrose, trehalose, dextrin, inulin, raffinose, starch, dulcitol, glycerol, mannitol, sorbitol, and inositol. It is non-hemolytic, does not liquefy gelatin, does not produce nitrites and nitrates and does not produce ammonia from urea. It gives a positive catalase test. The morphology, growth and staining characteristics indicate that it is closely related to *Corynebacterium bovis*.†

The culture was maintained on a yeast extract glucose agar. An inoculum was prepared by growing the organism at 25°C for 48 hours on the basal medium (Table I) plus refined liver extract. The cells were centrifuged, resuspended in an equal volume of saline and one drop used per 2 ml assay tube. The incubation temperature was 25°C. The organism was incapable of growing on the basal medium (Table I) unless natural supplements such as refined liver extract or yeast were added. It was then found that growth could be produced by a concentrate of protogen containing 20,000 units‡ per milligram of solids. The results (Table II) show that 0.02 unit of protogen, contained in 0.001 μg of solids, produced half maximum growth after 40 hours of incubation and almost maximum growth after 88 hours. It appeared that protogen was the only growth factor required as maximum growth was obtained with the protogen concentrate when a 40 or 88 hour incubation period was used.

Discussion. Since the completion of these experiments, Snell and Broquist(4) have suggested the identity of protogen with the "acetate factor" of Guirard *et al.*(5) These workers had previously demonstrated that small amounts of acetic acid function as a growth stimulant for lactic acid bacteria. Concentrates of natural materials are capable of replacing acetic acid and are more active per unit of weight than acetic acid itself. In the experiments of Snell and Broquist, concentrates of protogen were active for *Lactobacillus casei* as sources of the "acetate factor." This suggests a relationship between protogen and the metabolism of acetic acid in lactic acid organisms. While *L. casei* requires protogen only in the absence of acetate, both *T. geleii* and the corynebacterium described here need protogen even in the presence of sodium acetate. It has been reported, however, by Kidder and Dewey(6) that the

TABLE II.
Effect of Protogen on Growth of an Unidentified Corynebacterium.

Supplement per 2 ml medium	Growth (optical density) 40 hr	88 hr
None	.025	.07
.01 μl refined liver extract	.25	.47
.03 " " " "	.35	.76
.10 " " " "	.41	.90
1.0 " " " "	.67	1.25
.02 unit protogen*	.35	.77
.06 " "	.45	.95
.20 " "	.55	1.02
.60 " "	.65	1.05

* Supplied as a concentrate containing 20 units per μg of solids.

† We are indebted to Dr. A. Zink of Lederle Laboratories for classifying the organism and determining its morphological and cultural characteristics.

‡ One unit of protogen is the activity contained in one mg of a standard liver preparation.

4. Snell, E. E., and Broquist, H. P., *Arch. Biochem.*, 1949, v23, 326.

5. Guirard, B. M., Snell, E. E., and Williams, R. J., *Arch. Biochem.*, 1946, v9, 381.

growth rate of *T. geleii* can be increased by the addition of 1.0 mg per ml of sodium acetate.

The requirement of the different organisms for protogen differs widely. The requirements of *T. geleii,* the corynebacterium, and *L. casei* for protogen are respectively 0.5, 0.01 and 0.025 unit per ml for maximum growth. The protogen requirements for *L. casei* can be replaced by 0.25 mg of sodium acetate per ml.

Summary. An unidentified gram-positive bacillus has been found which requires protogen for growth in about one-fiftieth of the concentration required by *Tetrahymena geleii.*

6. Kidder, G. W., and Dewey, V., *Arch. Biochem.,* 1949, v20, 433.

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Applicability of a Differential Analyzer to Determination of Protein Fractions by the Electrophoretic Technic. (17976)

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This laboratory has found it advantageous to use a differential analyzer in place of a planimeter in connection with determination of concentrations of plasma protein fractions from electrophoretic patterns. The differential analyzer is an analogue computer developed at the Massachusetts Institute of Technology under the direction of Dr. Vannevar Bush. One was recently acquired by Wayne University. Its use was made available to us and the work was carried out at Wayne University Computation Laboratory under the direction of Prof. Arvid W. Jacobson.

Although the primary purpose of the analyzer is the solution of differential equations(1) the principles upon which it operates are quite simple. All quantities involved in the calculations are represented by the rotation of shafts. The fundamental unit is the integrator which is shown in Fig. 1. The disc A turns the wheel B by friction. Hence, a slight amount of rotation, ΔW of wheel B will be proportional to the product of the corresponding rotation, ΔV of disc A and the distance U of wheel B from the center of A; *i.e.,*

$$W = k \cdot U \Delta V$$

Thus, if u and v vary, the rotation of wheel B will be represented by the integral

$$W = k \int U dV.$$

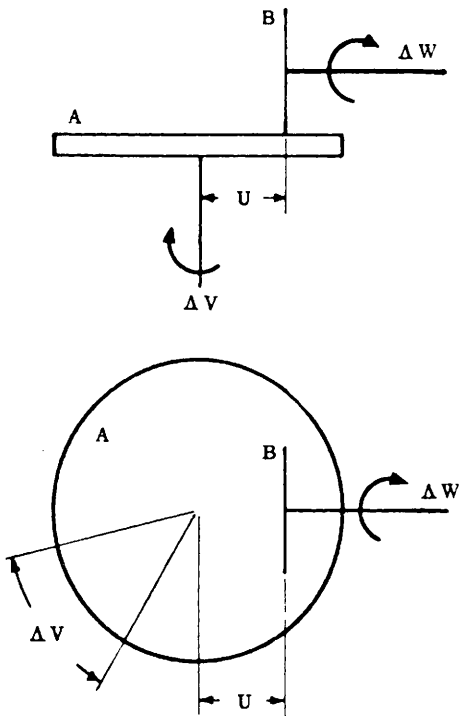

FIG. 1.
Integrator.

Fig. 2 shows a schematic diagram of the entire operation. In this drawing the mecha-

1. For a complete discussion of the differential analyzer see Bush, V., and Caldwell, S. H., *J. Franklin Inst.,* 1945, v240, 255.