centrations of the tissues with low I.A.A. treatments and of controls did not change appreciably during the growth period, but showed a definite, small increase with high I.A.A. treatments. The starch concentrations of the tissues were again relatively low, and in contrast with the results after 65 days growth in the first experiment, there was no increase from treatments with I.A.A.

Discussion and conclusions. The present results demonstrate a definite correlation between the effects of different concentrations of I.A.A. on growth and on reducing sugar concentrations in the tissues. To our knowledge this is the first case of such a relationship between a metabolic constituent and growth of tissues in response to treatments with an auxin.

We have no explanation for the contrast between these results and reports in the literature other than that differences in methods and plant materials may be responsible. The tobacco tissues we used have a low endogenous auxin content and they were analyzed after long growth periods with added I.A.A.; however definite increases in reducing sugar from treatments with I.A.A. may be obtained within the first week. Furthermore, the tissues were growing continuously and particularly those treated with I.A.A. continued to increase both in size and dry weight, whereas earlier tests have been performed on tissues capable of only limited growth in length and undergoing marked losses in dry weight regardless of the treatments. It should be pointed out that in the present work a correlation is demonstrated between increase in weight, rather than growth rate of the pieces, and the concentration of reducing sugar. This distinction may not be important but will be investigated. The reducing material has been expressed in terms of glucose but has not yet been characterized, and may not be simply glucose.

Conclusion. Auxin has a catalytic function in carbohydrate metabolism which is related to its effect on growth but which is not primarily concerned in the respiratory degradation of sugars. The above results are, therefore, reported at this time as they may be of general interest in studies of the mechanism of auxin action in the growth of plants.

Received May 31, 1950. P.S.E.B.M., 1950, v74.

Effect of Carbohydrate on Growth Response to Vitamin B_{12} in the Hyperthyroid Rat.* (17974)

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A rat assay for the estimation of vitamin B_{12} in biological materials previously reported (1) makes use of a corn-soybean meal basal ration containing iodinated casein. Ershoff (2), however, has noted that no increase in growth is obtained with vitamin B_{12} in rats fed thyroid active materials when a purified sucrose-casein diet is used. Whole liver, on the other hand, gives a maximum response. Betheil and Lardy(3) report that vitamin B_{12} will partially overcome the growth retardation in the hyperthyroid rat on the sucrose-casein ration. In his latest report Ershoff(4) demonstrates that full-fat soybean

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

1. Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, v177, 129.

2. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 209.

3. Betheil, J. J., and Lardy, H. A., *J. Nutrition*, 1949, v37, 495.

4. Ershoff, B. H., *J. Nutrition*, 1949, v39, 259.

TABLE I.
Comparison of Growth Response to Vitamin B_{12} in Rats on the Corn-Soybean Meal and Sucrose-Casein Rations.

Ration	Avg growth 3-wk period, g	Food consumption, g/rat/day
Corn-soybean basal (0.1% iodinated casein)	52	13.2
,, ,, ,, ,, ,, ,, + 0.2 μg B_{12}/day	110	—
Sucrose-casein basal (0.1% iodinated casein)	52	—
,, ,, ,, ,, ,, ,, + 0.2 μg B_{12}/day	69	—
Corn-soybean basal (0.1% iodinated casein) + 10% corn oil	61	10.4

meal has an antithyrotoxic effect and he correlates its effectiveness with the amount of fat present in the meal. Furthermore, with his sucrose-casein regime, soybean oil, as well as several other animal and vegetable fats, was capable of significantly ameliorating the decreased rate of growth in the hyperthyroid rat. The following work was undertaken to further elucidate the nature of these observed antithyrotoxic effects as they may be related to the specificity of the rat assay for vitamin B_{12}.

Experimental. Two basal rations were used. The corn-soybean basal diet was identical to that employed in the rat assay for vitamin B_{12} except that the level of most of the vitamins was doubled(1). Its composition is as follows: corn meal (5% fat) 45%, commercial soybean meal (5% fat) 45%, salt mixture† 2%, corn oil 5%, cystine 0.3%. The vitamins were added in the following amounts: choline chloride 100, thiamine hydrochloride 0.6, pyridoxine hydrochloride 0.4, calcium pantothenate 4.0, niacin 4.0, folic acid 0.05, biotin 0.02, p-amino-benzoic acid 25, riboflavin 0.6 and inositol 20 mg/100 g ration. In addition each rat received 2 drops of halibut liver oil weekly. In the sucrose-casein ration, sucrose 63% and casein‡ 18%, replaced the corn and soybean meals in the above diet but otherwise it was identical. Crystalline vitamin B_{12} when used was administered by injection. The desiccated thyroid§ and iodinated casein|| were mixed directly into the rations. Male weanling rats (Sprague-Dawley strain), within a weight range of 40 to 45 g at the start of an experiment, were employed. The average gain in weight during the first three weeks of growth was used as a measure of activity. Individual cages were used with feed and water being given *ad libitum.*

Results and discussion. Comparison of the growth promoting activity of vitamin B_{12} in rats on the sucrose-casein and the corn-soybean meal rations containing 0.1% iodinated casein is shown in Table I. On the corn-soybean diet, growth was approximately doubled over a 3-week period with vitamin B_{12} supplementation. Only slight improvement was noted upon addition of vitamin B_{12} to the sucrose-casein ration.

This partial growth stimulation to vitamin B_{12} with the diet containing sucrose and casein is in agreement with the results of Betheil and Lardy(3). A possible explanation for the difference in these data and those of Ershoff may lie in the nature of the rations used. The rats in the experiments being reported, and likewise those used by Betheil

† Salts IV. *J. Biol. Chem.*, 1935, v104, 657.

‡ Vitamin test casein, General Biochemicals, Inc., Chagrin Falls, O.

§ Thyroid powder, U.S.P., Wilson Laboratories, Chicago, Ill.

|| Supplied by Dr. W. R. Graham, Cerophyl Laboratories, Inc., Kansas City, Mo.

TABLE II.
Antithyrotoxic Effect of Soybean and Corn Meals in Sucrose Casein Ration Containing 0.5% Desiccated Thyroid.

Ration	Avg growth 3 wk period, g
Sucrose-casein basal	58
,, ,, ,, + 10% whole liver powder	88
,, ,, ,, + 1 μg B_{12}/day	66
,, ,, ,, + 45% corn meal + 1 μg B_{12}/day	94
,, ,, ,, + 45% soybean meal + 1 μg B_{12}/day	83

and Lardy, received 5% corn oil in their diet, while Ershoff employed a fat free basal ration and supplemented his animals with 500 mg cottonseed oil 3 times a week. This is significant in the light of Ershoff's report (4) that corn oil, as well as other animal and vegetable fats, exerts an antithyrotoxic effect. It was thought that the nature of this effect might possibly be attributed to food consumption. Two groups were fed the corn-soybean ration (0.1% iodinated casein) while one received an additional 10% corn oil. The growth and food consumption data are shown in Table I. The animals fed the increased amount of corn oil grew slightly better than those on the regular corn-soybean basal. Their food consumption, however, was less (20%). Therefore, by consuming less feed, less iodinated casein was ingested which could account for the growth response.

The possibility that corn meal might possess the antithyrotoxic activity found with soybean meal was next investigated. Corn meal and soybean meal were added individually at a 45% level to the sucrose-casein basal ration at the expense of the carbohydrate and protein of this diet. The total amount of protein was kept constant in the different rations. 0.5% desiccated thyroid and 1 μg per day of injected vitamin B_{12} were used. As shown in Table II, a response to vitamin B_{12} was observed when both the corn and soybean flours were used. The amount of corn meal needed to produce the stimulatory effect was then determined. From the data in Table III it is seen that at least 30% corn meal must be included in the diet to obtain maximum growth promotion when 0.3% iodinated casein is used. This amount of iodinated casein is approximately equivalent to 0.5% desiccated thyroid.

TABLE III.
Growth Response to Vitamin B_{12} of Rats on Casein-Sucrose Ration Containing 0.3% Iodinated-Casein Supplemented with Graded Levels of Corn Meal.

Supplement	Avg growth 3 wk period, g
None	48
0.2 μg B_{12}/day (inj.)	51
,, ,, ,, + 5% corn meal	52
,, ,, ,, + 10% ,, ,,	60
,, ,, ,, + 20% ,, ,,	60
,, ,, ,, + 30% ,, ,,	69
,, ,, ,, + 45% ,, ,,	69

The similar effectiveness of corn meal and soybean meal suggested that the nature of the carbohydrate in a diet may determine its antithyrotoxic activity. The validity of this idea was borne out by the following experiment. Dextrin and corn starch were substituted for the sucrose in the sucrose-casein ration. The data are presented in Table IV. The addition of either dextrin or corn starch counteracted the growth depression noted when sucrose served as the carbohydrate. Vitamin B_{12} produced a significant growth response when administered to the dextrin and corn starch regimes but only a slight increase in conjunction with the sucrose ration.

Defatted corn meal (Soxhlet extraction) when tested in the sucrose-casein basal ration at a level of 45% possessed activity equal to that of the dextrin and corn starch. The crude fat that was extracted produced some growth stimulation when included at a 2% level. These results are presented in Table IV.

The manner in which the carbohydrate of the diet functions to produce the growth stimulation noticed is not readily explained.

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.

Received May 31, 1950. P.S.E.B.M., 1950, v74.

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

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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.